

SUPPLEMENTAL TABLES

Table S1. Antimicrobial susceptibility testing of wild-type and isogenic deletion strains.

| Strain | PEN ^a | VAN | PMB | DAP | BAC | TET | ERY |
|----------------|--------------------|------|-----|-------|-------|------|------|
| MGAS10870 (WT) | 0.004 ^b | 0.25 | 24 | 0.094 | 0.25 | 0.50 | 0.25 |
| $\Delta liaR$ | 0.004 | 0.25 | 16 | 0.094 | 0.125 | 0.50 | 0.25 |
| $\Delta liaF$ | 0.004 | 0.50 | 16 | 0.094 | 0.38 | 0.38 | 0.19 |

^a Abbreviations: bacitracin (BAC), daptomycin (DAP), erythromycin (ERY), penicillin (PEN), polymyxin B (PMB), tetracycline (TET), vancomycin (VAN).

^b Values are micrograms per milliliter ($\mu\text{g}/\text{mL}$) and determined using E-test according to the protocol for beta-hemolytic streptococci and Clinical Laboratory Standards Institute (CLSI) (1). Values are from a single replicate but all samples were performed in at least biological duplicate with <2-fold difference in minimal inhibitory concentration.

Table S2. Differential gene expression of isogenic deletion mutants derived from RNA-sequencing.

| Locus tag ^a | Gene | Predicted Protein ^b | Log ₂ ΔLiaF/WT ^c | Log ₂ ΔLiaR/WT ^c |
|------------------------|-----------------|--|---|---|
| 10870_p61 | <i>speC</i> | Streptococcal pyrogenic exotoxin, C | -1.74 | 1.06 |
| spyM18_0779 | <i>mf2</i> | Mitogenic factor 2 | -1.66 | 0.94 |
| SpyM3_0010 | <i>mesJ</i> | cell-cycle protein | 1.62 | |
| SpyM3_0011 | <i>hpt</i> | hypoxanthine-guanine phosphoribosyltransferase | 1.74 | |
| SpyM3_0012 | <i>ftsH</i> | cell division protein | 1.85 | |
| SpyM3_0016 | <i>recO</i> | DNA repair protein RecO | 0.67 | |
| SpyM3_0019 | <i>purC</i> | phosphoribosylaminoimidazole-succinocarboxamide synthase | -1.24 | 0.80 |
| SpyM3_0020 | <i>purL</i> | phosphoribosylformylglycinamide synthase II | -1.24 | 0.95 |
| SpyM3_0021 | <i>purF</i> | amidophosphoribosyltransferase | -1.18 | 0.84 |
| SpyM3_0022 | <i>purM</i> | phosphoribosylaminoimidazole synthetase | -1.30 | 0.75 |
| SpyM3_0023 | <i>purN</i> | phosphoribosylglycinamide formyltransferase bifunctional | -1.07 | 1.13 |
| SpyM3_0024 | <i>purH</i> | phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase | -1.06 | 1.07 |
| SpyM3_0026 | <i>purD</i> | phosphoribosylamine--glycine ligase | -1.83 | 0.89 |
| SpyM3_0027 | <i>purE</i> | phosphoribosylaminoimidazole carboxylase catalytic subunit | -1.79 | 0.90 |
| SpyM3_0028 | <i>purK</i> | phosphoribosylaminoimidazole carboxylase ATPase subunit | -1.68 | 0.96 |
| SpyM3_0029 | | hypothetical protein | -0.90 | |
| SpyM3_0030 | <i>purB</i> | adenylosuccinate lyase | -0.63 | |
| SpyM3_0031 | | hypothetical protein | -0.89 | |
| SpyM3_0036 | <i>adh2</i> | bifunctional acetaldehyde-CoA/alcohol dehydrogenase | 1.41 | 0.78 |
| SpyM3_0037 | <i>adh1</i> | alcohol dehydrogenase | 0.84 | |
| SpyM3_0077 | | DNA binding protein | -1.80 | |
| SpyM3_0098 | <i>cbp/ap-1</i> | collagen binding protein; pilus accessory protein-1 | -1.02 | 2.14 |
| SpyM3_0099 | <i>srtA1</i> | Pilus, sortase A1 | -0.90 | 2.02 |
| SpyM3_0100 | <i>tee3/bp</i> | Pilus, backbone protein (tee) | -0.95 | 2.03 |
| SpyM3_0101 | <i>srtA2</i> | Pilus, sortase A2 | -0.92 | 1.71 |
| SpyM3_0102 | <i>ap-2</i> | Pilus, accessory protein-2 | -0.69 | 1.61 |
| SpyM3_0103 | <i>msmR</i> | multiple sugar metabolism regulator | -0.83 | |
| SpyM3_0105 | | hypothetical protein | 1.98 | -1.98 |
| SpyM3_0112 | | hypothetical protein | 0.87 | |
| SpyM3_0113 | | regulatory protein | 0.97 | |
| SpyM3_0118 | <i>ntpC</i> | V-type Na ⁺ -ATPase subunit C | 0.65 | |
| SpyM3_0123 | | toxic anion resistance protein | 0.64 | |
| SpyM3_0125 | <i>purA</i> | adenylosuccinate synthetase | 0.77 | |

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|------------|---------------|---|-------|-------|
| SpyM3_0128 | <i>nga</i> | NAD glycohydrolase | | -1.35 |
| SpyM3_0129 | <i>ifs</i> | hypothetical protein | | -1.40 |
| SpyM3_0130 | <i>slo</i> | streptolysin O | | -1.40 |
| SpyM3_0131 | | hypothetical protein | 1.76 | -3.12 |
| SpyM3_0132 | | hypothetical protein | 2.28 | -4.21 |
| SpyM3_0133 | <i>metB</i> | cystathionine beta-lyase | -1.03 | 2.08 |
| SpyM3_0141 | | transcriptional regulator | -0.61 | |
| SpyM3_0143 | <i>opuAA</i> | glycine betaine/proline ABC transporter | -0.89 | |
| SpyM3_0144 | <i>opuABC</i> | glycine-betaine binding permease protein | -0.74 | |
| SpyM3_0145 | <i>polA.1</i> | DNA polymerase I | 0.59 | |
| SpyM3_0150 | | hypothetical protein | -0.92 | |
| SpyM3_0151 | | biotin synthase | -0.84 | |
| SpyM3_0160 | <i>hasC.2</i> | UDP-glucose pyrophosphorylase | 0.64 | |
| SpyM3_0161 | <i>gpsA</i> | NAD(P)H-dependent glycerol-3-phosphate dehydrogenase | 0.63 | |
| SpyM3_0162 | | hypothetical protein | -1.25 | |
| SpyM3_0163 | | ABC transporter ATP-binding/permease protein | -0.95 | |
| SpyM3_0164 | | ABC transporter ATP-binding/membrane spanning protein | -0.88 | |
| SpyM3_0232 | <i>atmA</i> | ABC transporter substrate-binding protein | -0.73 | |
| SpyM3_0236 | <i>braB</i> | branched-chain amino acid transport protein | -1.22 | 0.68 |
| SpyM3_0237 | | serine/threonine transporter SstT | -1.24 | |
| SpyM3_0243 | | hypothetical protein | | -0.62 |
| SpyM3_0256 | | OxaA-like protein precursor | 1.63 | |
| SpyM3_0259 | | hypothetical protein | -0.66 | |
| SpyM3_0272 | | hypothetical protein | -0.88 | |
| SpyM3_0280 | <i>fhuG.1</i> | ferrichrome ABC transporter (permease) | | 0.64 |
| SpyM3_0281 | <i>fhuB</i> | ferrichrome ABC transporter (permease) | | 0.59 |
| SpyM3_0282 | <i>fhuD.1</i> | ferrichrome ABC transporter (ferrichrome-binding protein) | | 0.59 |
| SpyM3_0283 | <i>fhuC.1</i> | ferrichrome ABC transporter ATP-binding protein | | 0.65 |
| SpyM3_0285 | | polysaccharide biosynthesis protein | 0.69 | |
| SpyM3_0298 | <i>prtS</i> | IL-8 protease (PrtS/SpyCEP) | 1.63 | -4.17 |
| SpyM3_0299 | | hypothetical protein | -0.69 | 0.72 |
| SpyM3_0304 | | hypothetical protein | | -1.32 |
| SpyM3_0305 | | hypothetical protein | 0.79 | -2.32 |
| SpyM3_0306 | | hypothetical protein | 0.59 | |
| SpyM3_0307 | | hypothetical protein | | -1.24 |
| SpyM3_0331 | | hypothetical protein | | -0.76 |
| SpyM3_0333 | <i>phoH</i> | phosphate starvation-induced protein | 0.65 | |
| SpyM3_0334 | | hypothetical protein | 0.83 | |
| SpyM3_0346 | <i>mutR</i> | positive transcriptional regulator | | -0.77 |
| SpyM3_0349 | | ATPase | 0.59 | |

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|------------|---------------|---|-------|-------|
| SpyM3_0359 | <i>gloA</i> | lactoylglutathione lyase | 0.76 | -0.59 |
| SpyM3_0360 | | NAD(P)H-dependent quinone reductase | 0.71 | -0.64 |
| SpyM3_0361 | <i>pepQ</i> | Xaa-Pro dipeptidase | 0.77 | -0.63 |
| SpyM3_0363 | | sugar transferase | 2.53 | |
| SpyM3_0364 | | 1,2-diacylglycerol 3-glucosyltransferase | 2.68 | |
| SpyM3_0375 | <i>rnc</i> | ribonuclease III chromosome condensation and segregation SMC | 0.74 | |
| SpyM3_0376 | <i>smc</i> | protein | 0.73 | |
| SpyM3_0377 | | positive transcriptional regulator | -0.74 | |
| SpyM3_0393 | | hypothetical protein | 0.60 | |
| SpyM3_0394 | | hypothetical protein | 0.69 | |
| SpyM3_0402 | | drug resistance protein (transporter) PTS system beta-glucoside-specific transporter | | 0.99 |
| SpyM3_0404 | | subunit IIABC | | 0.79 |
| SpyM3_0406 | | hypothetical protein | 0.69 | -0.69 |
| SpyM3_0407 | | hypothetical protein | | -0.73 |
| SpyM3_0408 | | hypothetical protein | | -1.14 |
| SpyM3_0411 | | hypothetical protein | 2.62 | -1.78 |
| SpyM3_0428 | | glutathione peroxidase | 0.72 | |
| SpyM3_0429 | <i>pepF</i> | oligoendopeptidase F | 0.78 | |
| SpyM3_0437 | | hypothetical protein | 0.59 | |
| SpyM3_0458 | | acetoin reductase | 0.70 | |
| SpyM3_0466 | <i>adcA</i> | adhesion protein | | -0.78 |
| SpyM3_0467 | | GntR family transcriptional regulator | -0.69 | |
| SpyM3_0480 | <i>sagA</i> | Streptolysin S, precursor peptide | -0.78 | |
| SpyM3_0481 | <i>sagB</i> | Streptolysin S, putative dehydrogenase | -0.90 | 0.77 |
| SpyM3_0482 | <i>sagC</i> | Streptolysin S, putative cyclodehydratase | -0.85 | 0.73 |
| SpyM3_0483 | <i>sagD</i> | Streptolysin S, putative docking protein | -0.91 | 0.77 |
| SpyM3_0484 | <i>sagE</i> | Streptolysin S, putative immunity protein | -0.68 | 0.74 |
| SpyM3_0485 | <i>sagF</i> | Streptolysin S, putative membrane protein | -0.66 | 0.75 |
| SpyM3_0486 | <i>sagG</i> | Streptolysin S, putative ABC transporter | -0.80 | 0.80 |
| SpyM3_0487 | <i>sagH</i> | Streptolysin S, putative ABC transporter | -0.72 | 0.84 |
| SpyM3_0488 | <i>sagI</i> | Streptolysin S, putative ABC transporter | -0.62 | 0.74 |
| SpyM3_0489 | | hypothetical protein | -1.35 | |
| SpyM3_0513 | <i>rexB</i> | ATP-dependent nuclease subunit B | 0.65 | |
| SpyM3_0514 | <i>rexA</i> | ATP-dependent exonuclease subunit A | 0.65 | |
| SpyM3_0547 | <i>folC.2</i> | folyl-polyglutamate synthetase | 0.72 | |
| SpyM3_0548 | | hypothetical protein | 0.74 | -0.68 |
| SpyM3_0563 | | hypothetical protein | -0.70 | |
| SpyM3_0569 | | surface antigen | | -0.61 |
| SpyM3_0570 | | hypothetical protein | | -0.65 |
| SpyM3_0581 | | peptidoglycan hydrolase | 1.13 | -1.01 |
| SpyM3_0583 | <i>mac-1</i> | IgG protease (Mac-1/IdeS) | 1.57 | -3.39 |

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|------------|--------------|---|-------|-------|
| SpyM3_0591 | | 5'-nucleotidase | -1.15 | 1.16 |
| SpyM3_0602 | <i>dyr</i> | dihydrofolate reductase | 0.60 | |
| SpyM3_0614 | <i>cpsY</i> | transcriptional regulator | 0.73 | |
| SpyM3_0629 | | hypothetical protein | -1.59 | |
| SpyM3_0630 | | hypothetical protein | -1.70 | |
| SpyM3_0631 | | hypothetical protein | -0.97 | |
| SpyM3_0633 | | hypothetical protein | | -0.73 |
| SpyM3_0653 | | ABC transporter substrate-binding protein | -1.37 | |
| SpyM3_0655 | | ABC transporter membrane-spanning permease | -1.37 | |
| SpyM3_0656 | | ABC transporter ATP-binding protein | -1.28 | |
| | | PTS system mannose/fructose family transporter | | |
| SpyM3_0741 | | subunit IIA | -0.82 | |
| | | PTS system mannose/fructose transporter subunit IIB | | |
| SpyM3_0742 | | IIB | -0.68 | |
| SpyM3_0749 | <i>uvrC</i> | excinuclease ABC subunit C | 0.78 | -0.71 |
| SpyM3_0780 | | hypothetical protein | 0.63 | |
| | | oxidoreductase, short chain dehydrogenase/reductase | | |
| SpyM3_0788 | | oxidoreductase, short chain dehydrogenase/reductase | 0.84 | |
| SpyM3_0789 | | oxidoreductase, short chain dehydrogenase/reductase | 0.72 | |
| SpyM3_0814 | | hypothetical protein | 0.77 | |
| SpyM3_0816 | | hypothetical protein | 0.69 | |
| SpyM3_0821 | <i>gid</i> | tRNA (uracil-5-)-methyltransferase Gid | -0.94 | |
| SpyM3_0822 | | oxaloacetate decarboxylase | | 0.62 |
| SpyM3_0823 | | hypothetical protein | | 0.76 |
| | | citrate lyase synthetase (citrate (pro-3S)-lyase ligase) | | |
| SpyM3_0837 | <i>citC</i> | ligase) | 0.92 | -0.92 |
| SpyM3_0838 | | hypothetical protein | 0.90 | -1.01 |
| SpyM3_0848 | | hypothetical protein | -0.67 | 0.76 |
| SpyM3_0849 | <i>pdxK</i> | pyridoxamine kinase | -0.81 | 0.80 |
| SpyM3_0870 | | 16S rRNA m(2)G 1207 methyltransferase | -0.65 | |
| SpyM3_0871 | <i>coaA</i> | pantothenate kinase | 0.93 | |
| SpyM3_0873 | <i>ciaH</i> | sensor histidine kinase protein | | 0.65 |
| SpyM3_0877 | <i>pstB</i> | phosphate transporter ATP-binding protein | | 0.70 |
| SpyM3_0878 | <i>pstB2</i> | phosphate transporter ATP-binding protein | | 0.59 |
| SpyM3_0879 | <i>pstC</i> | phosphate ABC transporter (permease protein) | | 0.59 |
| SpyM3_0880 | <i>pstC2</i> | phosphate ABC transporter (permease protein) | | 0.63 |
| | | phosphate ABC transporter periplasmic phosphate-binding protein | | |
| SpyM3_0881 | <i>pstS</i> | phosphate-binding protein | | 0.62 |
| SpyM3_0902 | | hypothetical protein | -0.82 | |
| SpyM3_0905 | <i>cfa</i> | cAMP factor | -0.76 | 1.52 |
| SpyM3_0909 | | hypothetical protein | -0.65 | |
| SpyM3_0979 | | hypothetical protein | -0.66 | |
| SpyM3_0983 | <i>malE</i> | maltose/maltodextrin-binding protein | 0.68 | |

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|------------|---------------|--|-------|-------|
| SpyM3_0990 | | esterase | 0.84 | |
| SpyM3_0991 | <i>dltD</i> | secreted protein DltD | 0.78 | |
| SpyM3_0992 | <i>dltC</i> | D-alanine--poly(phosphoribitol) ligase subunit 2 dltB protein, D-alanine transfer from Dcp to undecaprenol-phosphate | 0.74 | |
| SpyM3_0993 | <i>dltB</i> | | 0.84 | |
| SpyM3_0994 | <i>dltA</i> | D-alanine--poly(phosphoribitol) ligase subunit 1 | 0.74 | |
| SpyM3_0997 | <i>glnP</i> | glutamine ABC transporter glutamine-binding protein/permease protein | 0.78 | |
| SpyM3_0998 | <i>glnQ</i> | ABC transporter ATP-binding protein - glutamine transport | 0.82 | |
| SpyM3_1008 | | hypothetical protein | -0.98 | |
| SpyM3_1019 | | hypothetical protein | 0.86 | |
| SpyM3_1020 | | hypothetical protein | | 1.38 |
| SpyM3_1032 | <i>grab</i> | protein G-related alpha 2M-bindingprotein | -0.90 | 1.05 |
| SpyM3_1044 | | peptidoglycan GlcNAc deacetylase | | -0.63 |
| SpyM3_1048 | <i>nrdH</i> | glutaredoxin-like protein | 0.83 | -0.72 |
| SpyM3_1049 | <i>nrdE.1</i> | ribonucleotide-diphosphate reductase subunit alpha | 0.94 | -0.59 |
| SpyM3_1050 | <i>nrdF</i> | ribonucleotide-diphosphate reductase subunit beta | 0.99 | |
| SpyM3_1061 | | oxalate:formate antiporter | -0.98 | |
| SpyM3_1068 | | hypothetical protein | | 1.13 |
| SpyM3_1070 | | hypothetical protein | -0.89 | 1.19 |
| SpyM3_1071 | <i>sodM</i> | superoxide dismutase | -0.74 | |
| SpyM3_1078 | <i>kup</i> | cation/potassium uptake protein | | 2.18 |
| SpyM3_1093 | <i>cadA</i> | heavy metal/cadmium-transporting ATPase | -0.81 | |
| SpyM3_1095 | <i>mf4</i> | mitogenic factor - phage associated | | -1.83 |
| SpyM3_1160 | | amino acid ABC transporter ATP-binding protein | -0.63 | 0.68 |
| SpyM3_1161 | | amino acid ABC transporter (permease protein) | | 0.73 |
| SpyM3_1186 | | ribose transport operon repressor | | 0.65 |
| SpyM3_1187 | | hypothetical protein | | 0.76 |
| SpyM3_1188 | <i>coaD</i> | phosphopantetheine adenylyltransferase | | 0.78 |
| SpyM3_1189 | | type II DNA modification methyltransferase | | 0.63 |
| SpyM3_1190 | <i>asnA</i> | asparagine synthetase AsnA | -1.35 | 1.61 |
| SpyM3_1191 | <i>arcC</i> | carbamate kinase | | 0.87 |
| SpyM3_1192 | | hypothetical protein | | 0.89 |
| SpyM3_1193 | | arginine repressor | | 0.83 |
| SpyM3_1194 | <i>arcB</i> | ornithine carbamoyltransferase | | 0.99 |
| SpyM3_1195 | | hypothetical protein | | 1.08 |
| SpyM3_1196 | <i>arcA</i> | arginine deiminase | | 1.05 |
| SpyM3_1267 | <i>msrA.1</i> | bifunctional methionine sulfoxide reductase A/B protein | 0.83 | |
| SpyM3_1268 | | hypothetical protein | 0.83 | |
| SpyM3_1269 | <i>ccdA</i> | cytochrome C-type biogenesis protein | 0.77 | |
| SpyM3_1272 | | hypothetical protein | 0.93 | |

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|------------|---------------|---|-------|-------|
| SpyM3_1285 | | beta-galactosidase | | 0.70 |
| SpyM3_1287 | | two-component sensor histidine kinase | | 0.60 |
| SpyM3_1301 | <i>speA3</i> | exotoxin type A precursor - phage associated | 1.43 | |
| SpyM3_1362 | | hypothetical protein | -0.65 | |
| SpyM3_1366 | | two-component response regulator | 0.80 | -8.31 |
| SpyM3_1367 | | two-component sensor histidine kinase | 0.73 | |
| SpyM3_1368 | | hypothetical protein | -7.60 | |
| SpyM3_1391 | <i>pepC</i> | aminopeptidase C | 0.72 | -0.65 |
| SpyM3_1409 | <i>sdn</i> | streptodornase (Sdn) - phage associated | -1.87 | |
| SpyM3_1469 | | hypothetical protein | -0.77 | |
| SpyM3_1473 | | hypothetical protein | | -0.59 |
| SpyM3_1478 | | hypothetical protein | 0.80 | |
| SpyM3_1493 | | esterase | 1.06 | -1.89 |
| SpyM3_1509 | | hypothetical protein | -1.25 | |
| SpyM3_1527 | <i>fabH</i> | 3-oxoacyl-ACP synthase | -0.73 | |
| SpyM3_1528 | | MarR family transcriptional regulator | -0.61 | |
| SpyM3_1557 | <i>cycD</i> | ABC transporter ATP-binding protein | | -0.74 |
| SpyM3_1558 | <i>fhuC.2</i> | ferrichrome transport ATP-binding protein | | -0.69 |
| SpyM3_1559 | <i>fhuG.2</i> | ferrichrome ABC transporter (permease) | | -0.60 |
| SpyM3_1566 | <i>pmi</i> | mannose-6-phosphate isomerase | 1.05 | -0.99 |
| SpyM3_1583 | | hypothetical protein | 0.89 | -1.27 |
| SpyM3_1584 | <i>mutY</i> | A/G-specific adenine glycosylase | | -0.83 |
| SpyM3_1599 | | hypothetical protein | -0.63 | |
| SpyM3_1614 | | GntR family transcriptional regulator | 0.70 | |
| SpyM3_1625 | <i>lppC</i> | acid phosphatase | | 0.63 |
| SpyM3_1645 | <i>salR</i> | response regulator of salavaricin regulon | 0.59 | |
| SpyM3_1646 | <i>salk</i> | two-component sensor histidine kinase | 0.62 | |
| SpyM3_1655 | <i>lacF</i> | PTS system lactose-specific transporter subunit IIA | -0.62 | |
| SpyM3_1656 | <i>lacD.2</i> | tagatose 1,6-diphosphate aldolase | -0.66 | |
| SpyM3_1657 | <i>lacC.2</i> | tagatose-6-phosphate kinase | -0.64 | |
| SpyM3_1666 | | hypothetical protein | -0.59 | |
| SpyM3_1687 | <i>polC</i> | DNA polymerase III PolC | 0.59 | |
| SpyM3_1698 | <i>ska</i> | streptokinase A precursor | 1.09 | -2.91 |
| SpyM3_1699 | | hypothetical protein | 1.21 | |
| SpyM3_1712 | <i>pabP</i> | para-aminobenzoate synthetase | 0.68 | |
| SpyM3_1714 | | recombination factor protein RarA | 0.63 | |
| SpyM3_1718 | <i>dppA</i> | surface lipoprotein DppA | 0.76 | -1.22 |
| SpyM3_1719 | <i>dppB</i> | dipeptide ABC transporter permease protein | 0.93 | -0.96 |
| SpyM3_1720 | <i>dppC</i> | dipeptide ABC transporter permease protein | 1.00 | -0.80 |
| SpyM3_1721 | <i>dppD</i> | dipeptide ABC transporter ATP-binding protein | 0.97 | -1.01 |
| SpyM3_1722 | <i>dppE</i> | dipeptide ABC transporter ATP-binding protein | 1.02 | -1.12 |
| SpyM3_1723 | | hypothetical protein | | -1.49 |

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|------------|--------------|---|-------|-------|
| SpyM3_1726 | <i>scpA</i> | C5A peptidase precursor | 0.88 | -2.63 |
| SpyM3_1727 | <i>emm3</i> | antiphagocytic M protein, type 3 | | -0.88 |
| SpyM3_1728 | <i>mga</i> | M protein trans-acting positive regulator | 0.70 | -1.11 |
| SpyM3_1730 | | hypothetical protein | | 0.70 |
| SpyM3_1731 | <i>isp.1</i> | hypothetical protein | | 0.64 |
| SpyM3_1732 | | histidine kinase | | 0.69 |
| SpyM3_1733 | | two-component response regulator | | 0.70 |
| SpyM3_1734 | | ABC transporter permease | | 1.41 |
| SpyM3_1735 | | ABC transporter ATP-binding protein | | 1.87 |
| SpyM3_1736 | | ATP-binding cassette transporter protein | | 2.09 |
| SpyM3_1737 | | hypothetical protein | | 2.42 |
| SpyM3_1738 | | hypothetical protein | 0.88 | -0.79 |
| SpyM3_1739 | | hypothetical protein | 1.01 | -1.97 |
| SpyM3_1740 | <i>prsA</i> | foldase protein PrsA | -1.19 | 1.26 |
| SpyM3_1741 | | hypothetical protein | | 8.67 |
| SpyM3_1742 | <i>speB</i> | pyrogenic exotoxin B | | 8.75 |
| SpyM3_1743 | | hypothetical protein | | 8.15 |
| SpyM3_1744 | <i>ropB</i> | transcription regulator | -0.82 | 1.25 |
| SpyM3_1745 | <i>mf</i> | mitogenic factor 25K precursor | -0.84 | |
| SpyM3_1747 | <i>gldA</i> | glycerol dehydrogenase | 0.67 | |
| SpyM3_1748 | <i>mipB</i> | fructose-6-phosphate aldolase | 0.70 | |
| SpyM3_1749 | <i>pflD</i> | pyruvate formate-lyase | 0.65 | |
| SpyM3_1758 | <i>pbp2A</i> | penicillin-binding protein 2a | 0.76 | |
| SpyM3_1759 | | translation initiation inhibitor | 0.79 | -2.09 |
| SpyM3_1784 | <i>pepO</i> | endopeptidase O | 1.08 | -0.97 |
| SpyM3_1785 | <i>dexS</i> | dextran glucosidase | | 0.72 |
| SpyM3_1786 | | PTS system transporter protein II | | 0.66 |
| SpyM3_1799 | <i>spxA</i> | transcriptional regulator Spx | 2.29 | -3.59 |
| SpyM3_1810 | | hypothetical protein | 0.79 | -0.85 |
| SpyM3_1826 | | hypothetical protein | -0.65 | |
| SpyM3_1841 | <i>sdhB</i> | L-serine dehydratase beta subunit | 0.98 | |
| SpyM3_1842 | <i>sdhA</i> | L-serine dehydratase alpha subunit | 0.92 | |
| SpyM3_1843 | | hypothetical protein | -1.06 | |
| SpyM3_1849 | | hypothetical protein | 0.77 | -0.80 |
| SpyM3_1850 | | hypothetical protein | 0.69 | -0.80 |
| SpyM3_1851 | <i>hasA</i> | hyaluronan synthase | 0.79 | -3.96 |
| SpyM3_1852 | <i>hasB</i> | UDP-glucose 6-dehydrogenase | 0.75 | -3.98 |
| SpyM3_1853 | <i>hasC</i> | UDP-glucose pyrophosphorylase | 0.75 | -3.82 |
| SpyM3_1857 | <i>guaB</i> | inosine 5'-monophosphate dehydrogenase | -0.71 | |
| SpyM3_1858 | <i>trsA</i> | tryptophanyl-tRNA synthetase | -0.92 | |
| SpyM3_1859 | | hypothetical protein | -0.79 | |
| SpyM3_1864 | <i>degP</i> | serine protease | | 0.69 |

^a Locus tag derived from MGAS315 (AE014074.1).

^b Predicted protein product as annotated in MGAS315 genome (AE014074.1).

^c Log₂ fold-change in mutant relative to wild-type (MGAS10870). Shown are only those genes that met criteria defined for significant differential gene expression as described in Methods.

Table S3. Bacterial strains used in this study.

| Strain | Description | Source/Reference |
|----------------------------|---|-------------------------|
| NEB5alpha | <i>E. coli</i> cloning strain | New England BioLabs |
| MGAS10870 | Wild-type serotype M3 group A <i>Streptococcus</i> | (2) |
| $\Delta liaF$ | MGAS10870 with in-frame, unmarked deletion in <i>liaF</i> | (3) |
| $\Delta liaR$ | MGAS10870 with in-frame deletion of <i>liaR</i> using <i>aad9</i> ($\Delta liaR::aad9$) | (3) |
| $\Delta spxA2$ | MGAS10870 with in-frame deletion of <i>spxA2</i> using <i>aad9</i> ($\Delta spxA2::aad9$) | This study |
| $\Delta ropB$ | MGAS10870 with in-frame deletion of <i>ropB</i> using <i>aph</i> ($\Delta ropB::aph$) | (3) |
| $\Delta clpX$ | MGAS10870 with in-frame deletion of <i>clpX</i> using <i>aad9</i> ($\Delta clpX::aad9$) | This study |
| $\Delta liaR/\Delta ropB$ | MGAS10870 with in-frame deletion of both <i>liaR</i> ($\Delta liaR::aad9$) and <i>ropB</i> ($\Delta ropB::aph$) | This study |
| $\Delta liaF/\Delta spxA2$ | MGAS10870 with in-frame, unmarked deletion of <i>liaF</i> and in-frame deletion of <i>spxA2</i> ($\Delta spxA2::aad9$) | This study |
| $\Delta liaR/\Delta spxA2$ | MGAS10870 with in-frame deletion of <i>liaR</i> using <i>aad9</i> ($\Delta liaR::aad9$) and <i>spxA2</i> using <i>aph</i> ($\Delta spxA2::aph$) | This study |

Table S4. Plasmids, primers, and probes used in this study.

| Primer | Sequence (5'-3') | Use |
|---------------|--|---|
| tufA-F | CAACTCGTCACTATGCGCACAT | qRT PCR; Forward primer for <i>tufA</i> |
| tufA-R | GAGCGGCACCAGTGATCAT | qRT PCR; Reverse primer for <i>tufA</i> |
| tufA-P | [6FAM]CTCCAGGACACGCGGACTACGTTAAAA[BHQ1] | qRT PCR; Probe for <i>tufA</i> |
| hasA-F | ACCGTTCCTTGCAATAAAGG | qRT PCR; Forward primer for <i>hasA</i> |
| hasA-R | CGTCAGCGTCAGATCTTTCAA | qRT PCR; Reverse primer for <i>hasA</i> |
| hasA-P | [6FAM]CGCCATGCTCAAGCGTGGGC[BHQ1] | qRT PCR; Probe for <i>hasA</i> |
| mac1-F | ATCGAACACCAGTGAAGCAT | qRT PCR; Forward primer for <i>mac1</i> |
| mac1-R | ACATTCAATGGAAAAGACGATCTTCT | qRT PCR; Reverse primer for <i>mac1</i> |
| mac1-P | [6FAM]TTCCTGGTGTGGCAGCCCC[BHQ1] | qRT PCR; Probe for <i>mac1</i> |
| spxA2-F | AAGGATGCGCGGGTTATCTT | qRT PCR; Forward primer for <i>spxA2</i> |
| spxA2-R | ATGCCAAAGCTCTCGATTGC | qRT PCR; Reverse primer for <i>spxA2</i> |
| spxA2-P | [6FAM]TCAAATCAATAACCTCACTAACACTT[BHQ1] | qRT PCR; Probe for <i>spxA2</i> |
| ska-F | ACTGATCGAAACGGCAAGGT | qRT PCR; Forward primer for <i>ska</i> |
| ska-R | TTCTTGACAGGTTGGGTGC | qRT PCR; Reverse primer for <i>ska</i> |
| ska-P | [6FAM]TGCTGACAAAGATGGTTCGGT[BHQ1] | qRT PCR; Probe for <i>ska</i> |
| grab-F | GCATCAGTATTAGTCGGTTCAACAGT | qRT PCR; Forward primer for <i>grab</i> |
| grab-R | GGTCCGCCATTTGGAATAA | qRT PCR; Reverse primer for <i>grab</i> |
| grab-P | [6FAM]TGTTGACTCACCTATCGAACAGCCTCGA[BHQ1] | qRT PCR; Probe for <i>grab</i> |
| tee3-F | AGCGTGACACTCAAGTTCCA | qRT PCR; Forward primer for <i>tee3</i> |
| tee3-R | TCCACCAATAGCCACAATGCT | qRT PCR; Reverse primer for <i>tee3</i> |
| tee3-P | [6FAM]ACCCTTGCTCCATTTGCAGT[BHQ1] | qRT PCR; Probe for <i>tee3</i> |
| speB-F | TGCCTACAACAGCACTTTGG | qRT PCR; Forward primer for <i>speB</i> |
| speB-R | GTGGAGTCTCTGACGGCTTCT | qRT PCR; Reverse primer for <i>speB</i> |
| speB-P | [6FAM]GCCTGCGCCGCCACCAGTA[BHQ1] | qRT PCR; Probe for <i>speB</i> |
| spxA2-A-F | AGCAGAACTTAGGTTAACTGAT | ChIP qRT PCR, Forward primer for <i>spxA2</i> motif A |
| spxA2-A-R | TTATCTTGGTAAAAATAAGGTTTT | ChIP qRT PCR, Forward primer for <i>spxA2</i> motif A |
| spxA2-A-P | [6FAM]ACTAATAATTCAAGGGAATACAGGTGA [BHQ1] | ChIP qRT PCR, probe for <i>spxA2</i> motif A |
| spxA2-B-F | GGTGAAAAACCTTATTTACCAAGA | ChIP qRT PCR, Forward primer for <i>spxA2</i> motif B |
| spxA2-B-R | TGCTGACTCACTTTCTATTTCTGTG | ChIP qRT PCR, Forward primer for <i>spxA2</i> motif B |

| | | |
|-----------|---|---|
| spxA2-B-P | [6FAM]AAGAAACTGTGCTACAATAAAGCT[BHQ1] | ChIP qRT PCR, probe for spxA2 motif B |
| MSP378 | GTACCATGCGCAGGATAAAACAC | Pair with MSP381 to generate 5' flanking region for <i>clpX::aad9</i> gene replacement |
| MSP381 | CTTTATTAATTTGTTTCGTATGTATTCAATTCTTATAATTCTCTTA CTTTCTCTTATC | Pair with MSP378 to generate 5' flanking region for <i>clpX::aad9</i> gene replacement |
| MSP379 | CTATAAAAGTATCAGATTTGTC | Pair with MSP382 to generate 3' flanking region for <i>clpX::aad9</i> gene replacement |
| MSP382 | CTATTTAAATAACAGATTAATAAAATTATAAGGAGAAATAATGG CTGAAGAACAAG | Pair with MSP379 to generate 3' flanking region for <i>clpX::aad9</i> gene replacement |
| MSP380 | GATAAGAGAAAGTAAGAGAATTATAAGAATTGAATACATACGA ACAAATTAATAAAG | Pair with MSP383 to generate <i>aad9</i> gene replacement <i>clpX::aad9</i> |
| MSP383 | CTTGTTCCTTCAGCCATTATTTCTCCTTATAATTTTTTAATCTGT TATTTAAATAG | Pair with MSP380 to generate <i>aad9</i> gene replacement <i>clpX::aad9</i> |
| 3122F | AAGCCAAGAGGATCACTAAT | Pair with MSP337 to generate 5' flanking region for <i>spxA2::aad9</i> gene replacement |
| MSP337 | ACTATTTAAATAACAGATTAATAAAATTATAAAAGCTCTTGTAG AGCTTTTTTTAAATACC | Pair with 3122F to generate 5' flanking region for <i>spxA2::aad9</i> gene replacement |
| MSP336 | GGTATTTAAAAAAGCTCTACAAGAGCTTTTATAATTTTTTTAA TCTGTTATTTAAATAGT | Pair with MSP339 to generate <i>aad9</i> gene replacement <i>spxA2::aad9</i> |
| MSP339 | CTATCACGAAATAGAAAGTGAGTCAGCAACTTGAATACATACG AACAAATTAATAAAG | Pair with MSP336 to generate <i>aad9</i> gene replacement <i>spxA2::aad9</i> |
| 3123R | CGGAGCGACCTTGTTTTAA | Pair with MSP338 to generate 3' flanking region for <i>spxA2::aad9</i> gene replacement |
| MSP338 | CTTTATTAATTTGTTTCGTATGTATTCAAGTTGCTGACTCACTTT CTATTTTCGTGATAG | Pair with 3123R to generate 3' flanking region for <i>spxA2::aad9</i> gene replacement |

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