

# Supplementary Figure 1.

*Streptococcus suis* . . . . . MSA I I E L K D V N L A Y T A S Q T M A L E D V N L V I P . R G S R T A I V G P N G A G K S S L F K V I L  
*Staphylococcus aureus* . . . . . M L E T K D L N L F L G . N K H V L K N I S L S I P V R G E I I G I M G P N G A G K S S L I K S L I  
*Enterococcus faecalis* . . . . . M R K S F N L A V Q A L T V Q Y Q G . R . T A L N N I H V T I P . S G K I I G I I G P N G A G K S S F I K G L L  
*Streptococcus pyogenes* M F Y L C K G D K M I T T N N L C V T Y D G . N S N A L E A I N V T I D . G P S I V G I I G P N G A G K S S F M K A I L  
*Streptococcus pneumoniae* . . . . . M I R I E N L S V S Y K E . T . L A L K D I S L V L H . G P T I I G I I G P N G A G K S S L K G M L  
*Streptococcus oligofermentans* . . . . . M I E M K N L S V S Y Q G . Q . L A L E P T S L T I K . G P T I I G I I G P N G A G K S S L I K G M L

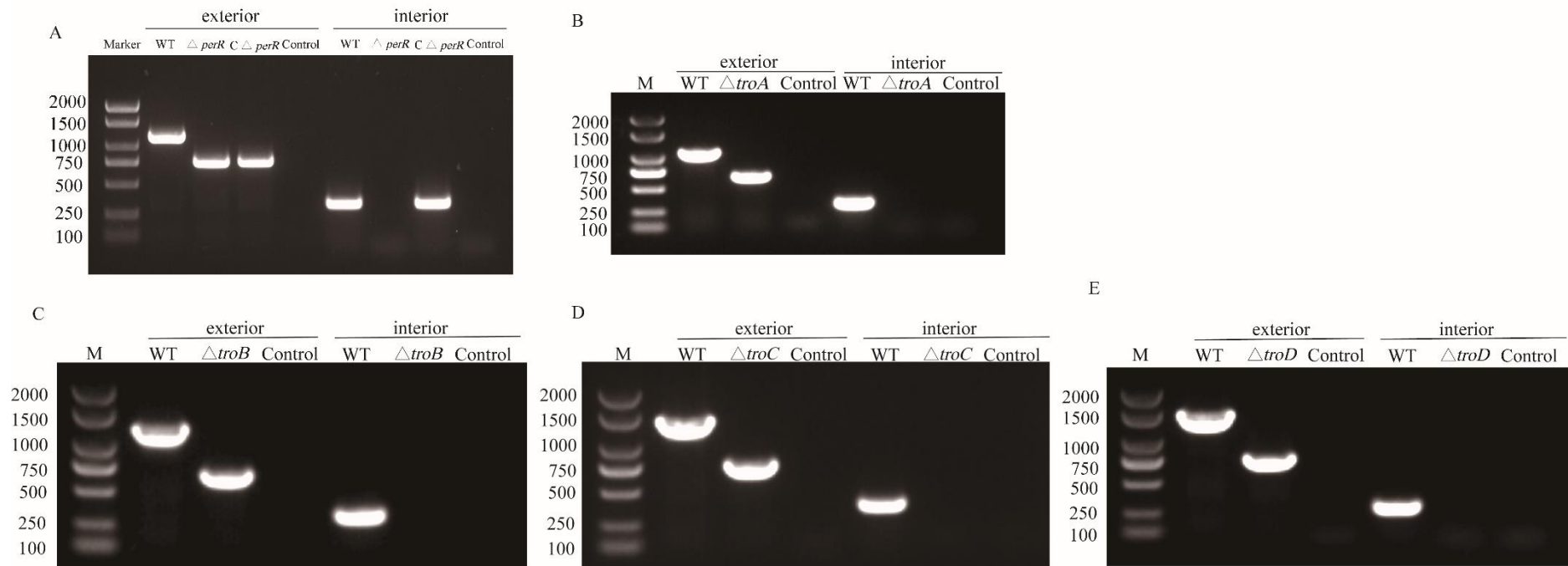
*Streptococcus suis* G L E K P D T G Q V R L L G Q E A G L E R L I A O K V A Y I P Q S S Q V N W Q F P A T V F E I V L M G R F A H S K G M F  
*Staphylococcus aureus* G E F N A T G . T K L L Y N K P I . . . Q Q L O H I T Y I P O K A H I L D L D F P S V E Q V I L S G C Y K . E I G W F  
*Enterococcus faecalis* G L I K T K E R D V L L N N Q A I . . . D Q Q T T I A Y V E O R S A L D L S F P S V F E T V L L G T Y P . N L G L L  
*Streptococcus pyogenes* N L I D Y Q G . H V T V D G K D G . . . R K L G H T V A Y V E O R S M I D Y N F P T V K E C V A L G T Y S . K L G L F  
*Streptococcus pneumoniae* G T I P H Q G . Q A F L D D K E V . . . K K S L H R I A Y V E O K I N I D Y N F P I K V K E C V S L G L F P . S I P L F  
*Streptococcus oligofermentans* G I V E S E G . Q T F L D R K P M . . . K Q E L S K I A Y V E O K I H I D Y N F P I K V K E C V S L G L F P . K I K L F

*Streptococcus suis* R R P T K A D R Q I V E Q A L E R L K T A D L R H R O I D Q L S G G Q R Q R V F I A R A L A Q E A E L Y L M D E P F A G  
*Staphylococcus aureus* R R P N K S A R D K L K Q L L S D L E L E S L R H R O I S E L S G G Q L Q R V L I A R A L M S E S E V Y F L D E P F V G  
*Enterococcus faecalis* K R P G K K E K Q A A M A A I K M V Q L E D Y A O R O I G E L S G G Q L Q R V F I A R V L A Q G A E V I F L D E P F V G  
*Streptococcus pyogenes* R R V G K K Q F E Q V D K V I K Q V G L E D F G H R P I K S L S G G Q E Q R M L V A R C L I Q E S D Y I F L D E P F V G  
*Streptococcus pneumoniae* R S L K A K H W K K V Q E A L E I V G L A D Y A B R O I S Q L S G G Q E Q R V L I A R C L V Q E A D Y I F L D E P F A G  
*Streptococcus oligofermentans* Q R L K A S D W E K V A R A L K I V G L E D F A B R O I S Q L S G G Q E Q R V L I A R C L V Q E A E Y I F L D E P F V G

*Streptococcus suis* I D Q A T E I M I M D M L K E F Q C E G K T S I V I H H D L T L D A Y F D H L V W I H K H V I D S G P M D E T L T S E  
*Staphylococcus aureus* I D F S S E K I T M T K I E N L K Q O G K L I L I I H H D L S K A K Q Y F D R I I L L N Q T L R Y F G D S E E A M S V I  
*Enterococcus faecalis* I D M S S E K V I M D I L K S L K N O G K M I I I V H H D L H K V S H Y F D E L I V I K N R L I A A G P V E Q T F T A E  
*Streptococcus pyogenes* I D S V S E K I T I V D L R K E L K M A G K T I L I V H H D L S K V E H Y F D K L M I L N K H L V A Y G N V C E V F T V D  
*Streptococcus pneumoniae* I D S V S E E I T M N T L R D L K K A G K T V L I V H H D L S K I P H Y F D Q V L L V N R E V I A F G P T K E T F T E T  
*Streptococcus oligofermentans* I D S V S E E I T M E T L R Q L R K A G K T I L I V H H D L S K V A K F D Q V L L L N K E V I A F G S T E S T F T K E

*Streptococcus suis* N Y Q A T Y G I G N G L F L G N A K G G S H V  
*Staphylococcus aureus* R L N E T F M S S T D C S D F S Q R S N I T C  
*Enterococcus faecalis* T L Q E A Y G D L L G D L L I Q G . V A K . .  
*Streptococcus pyogenes* T L S K A Y G N H L I . . . L G K . E M V . .  
*Streptococcus pneumoniae* N L K E A Y G N Q L F . . . F N G . G D L . .  
*Streptococcus oligofermentans* N M Q K T Y G S Q L F . . . M N G . G A . . .

Supplementary Figure 2.



Supplementary Table 1. Bacterial strains and plasmids used in this work.

| Strain or Plasmid           | Description <sup>a</sup>                              | Source or Reference |
|-----------------------------|---|---------------------|
| Strains                     |   |                     |
| SC19                        | From the brain of a dead pig                          | Our laboratory      |
| $\Delta troA$               | Deletion mutant of <i>troA</i> with SC19 background   | This study          |
| $\Delta troB$               | Deletion mutant of <i>troB</i> with SC19 background   | This study          |
| $\Delta troC$               | Deletion mutant of <i>troC</i> with SC19 background   | This study          |
| $\Delta troD$               | Deletion mutant of <i>troD</i> with SC19 background   | This study          |
| $\Delta perR$               | Deletion mutant of <i>perR</i> with SC19 background   | This study          |
| $C\Delta perR$              | Complemented strain of <i>perR</i> , $Spc^r$          | This study          |
| <i>E. coli</i> DH5 $\alpha$ | Cloning host for maintaining the recombinant plasmids | TAKARA              |
| <i>E. coli</i> BL21 (DE3)   | Host for expressing the recombinant proteins          | WEIDI               |
| Plasmids                    |   |                     |
| pSET4S                      | Temperature-sensitive suicide vector, $Spc^r$         | (1)                 |
| pSET2                       | vector for <i>S. suis</i> complementation, $Spc^r$    | (2)                 |
| pET-30a (+)                 | vector for protein expression, $Kan^r$                |                     |

<sup>a</sup>  $Spc^r$ , spectinomycin resistant;  $Kan^r$ , kanamycin resistant.

1. Takamatsu, D., Osaki, M., Sekizaki, T., 2001. Thermosensitive suicide vectors for gene replacement in *Streptococcus suis*. *Plasmid* 46, 140-148.
2. Takamatsu, D., Osaki, M., Sekizaki, T., 2001. Construction and characterization of *Streptococcus suis*-*Escherichia coli* shuttle cloning vectors. *Plasmid* 45, 101-113.

Supplementary Table 2.

| Primer              | Sequence (5'-3')  | Description  |
|---------------------|---|--|
| troA-upstream-F/R   | AAAAC TGCAGGAGA ACTCGCTTTCTTCTGAAACTA<br>GAGGAGCATAAAGAGTGAAGTCATATGGCTACATACAAATGAGGGTCTTC | Amplification of <i>troA</i> upstream homology arm   |
| troA-downstream-F/R | GAAGACCCTCATTGTATGTAGCCATATGACTTCACTCTTTATGCTCCTC<br>CGCGGATCCCTATTTTCAGATATTTAACCATCAGT    | Amplification of <i>troA</i> downstream homology arm |
| troA-exterior-F/R   | GAGAACTCGCTTTCTTCTGAAACTA<br>CTATTTTCAGATATTTAACCATCAGT                                     | Detection exterior of <i>troA</i> mutant strain      |
| troA-interior-F/R   | CTATCACGGTTTGCAC TTTGAAGGT<br>AAGGCATCATGTGGTGTTACCAAAT                                     | Detection interior of <i>troA</i> mutant strain      |
| troB-upstream-F/R   | AAAAC TGCAGCCCAAGCAAAACAGAATGCGATAGC<br>CGGCTATTGTTGGACCAAATGGTGC GATGAGACTTTGACAAGCGAGAA   | Amplification of <i>troB</i> upstream homology arm   |
| troB-downstream-F/R | TTCTCGCTTGTCAAAGTCTCATC GCACCATTTGGTCCAACAATAGCCG<br>CGCGGATCCAGATGATTTGCATGCCTGGGTAGAA     | Amplification of <i>troB</i> downstream homology arm |
| troB-exterior-F/R   | CCAAACCAAAGAAAGAAGCAGACAC<br>GTATAGGGTTTGCCAGCAATTTGTT                                      | Detection exterior of <i>troB</i> mutant strain      |
| troB-interior-F/R   | TGAAGCCAGACAAGGTGATCAAAGT<br>CGCTCATAGTAAGGGAATGTTTAGAAGA                                   | Detection interior of <i>troB</i> mutant strain      |
| troC-upstream-F/R   | AAAAC TGCAGACCTGTTCTTGGCTTATCATCTGGA<br>TCTTGGGCATGCTTCTTATCCTGGATTTGTCGACCGGACCGACTATTATC  | Amplification of <i>troC</i> upstream homology arm   |
| troC-downstream-F/R | GATAATAGTCGGTCCGGTTCGACAAA TCCAGGATAAGAAGCATGCCCAAGA<br>CGCGGATCCCATACGATTGAAGGAAGCATTGGTG  | Amplification of <i>troC</i> downstream homology arm |
| troC-exterior-F/R   | ACAATAACCAACCAAGCCAAACTCG   | Detection exterior of <i>troC</i> mutant strain      |

|                     |   |  |
|---------------------|---|--|
| troC-interior-F/R   | GATGAGACTTTGACAAGCGAGAA<br>ATTCCTGCCAGTAATAGACTCTTGT<br>TTCTTTGGTTTGGGAATGGTCTTGA   | Detection interior of <i>troC</i> mutant strain                        |
| troD-upstream-F/R   | AAAACCTGCAGTAGGCTGCTTTTTCTTGGTTTTGCG<br>CGGCTCGATACTGGTTGTAAAAAAT TTGTCAGCCTTCTTGTGGTTTTGTTT                              | Amplification of <i>troD</i> upstream homology arm                     |
| troD-downstream-F/R | GAACAAACCACAAGAAGGCTGACAA ATTTTTTACAACCAGTATCGAGCCG<br>CGCGGATCCATGGATGAGACTTTGACAAGCGAGA                                 | Amplification of <i>troD</i> downstream homology arm                   |
| troD-exterior-F/R   | AAACGGTCACGGATTCATAGTCAG<br>CAAGGGTTATTGGTGTTCGTGT  | Detection exterior of <i>troD</i> mutant strain                        |
| troD-interior-F/R   | GGCACAGGTCCCTGACATGGTTAAA<br>TGCGTGGGCTTACCATCGTTTA   | Detection interior of <i>troD</i> mutant strain                        |
| qPCR-troA-F/R       | TCCGGTCTTCTCAAAGCTT<br>GTCCATCGCAAACCACAGAA   | Detection the transcription of <i>troA</i> used in qRT-PCR             |
| qPCR-troB-F/R       | GGTCGCCTGATAATTCTCGC<br>TGGCACAGGAAGCAGAATTG  | Detection the transcription of <i>troB</i> used in qRT-PCR             |
| qPCR-troC-F/R       | AGAGGATGTCGCGACTGAAA<br>AGTTGCTTTAGGGACCGTCA  | Detection the transcription of <i>troC</i> used in qRT-PCR             |
| qPCR-troD-F/R       | GCCAAACTCGACTTGACCAG<br>GCCATTGACCGTCTGCATAG  | Detection the transcription of <i>troD</i> used in qRT-PCR             |
| 16SrRNA-F/R         | GTTGCGAACGGGTGAGTAA<br>TCTCAGGTCGGCTATGTATCG  | An internal region of 16S rRNA   |
| EMSA-troA-F/R       | CAGTTGACAAAATGATTTTTTTAGATTAATCTAATTATATAGTTAGGTATGCTTAATAA<br>TTATTAAGCATACCTAACTATATAATTAGATTAATCTAAAAAATCATTTTGTCAACTG | Amplification of promoter region of <i>troABCD</i> used in EMSA        |
| EMSA-control-F/R    | CGTGAAAAGGGGGTTACGACACCAGTTCTGATTACAACAGCCAAGGAAAGTCTAGAGGA<br>TCCTCTAGACTTTCTTGGCTGTTGTAATCAGAACTGGTGTGTAACCCCTTTTCACG   | Amplification of gene segment region used for negative control in EMSA |

|                        |   |   |
|------------------------|---|---|
| perR-upstream-F/R      | AAAAC TGCAGCAAGGAGAAGCGATGGTTAAGAGAA<br>ACGAAAGGAGGAAACTATGGA ACTCCCAAAGAGGTGCTATCCATTTACGG | Amplification of <i>perR</i> upstream homology arm.                 |
| perR-downstream-F/R    | CCGTAAATGGATAGCACCTCTTTGGGAGTTCCATAGTTTCCTCCTTTTCGT<br>CGCGGATCCGCCGCAGTCGGACCCGCCAAACCAC   | Amplification of <i>perR</i> downstream homology arm.               |
| perR-exterior-F/R      | GATAGCAGCTAAAATCATGAGCAGG<br>CTGGCTGACCACTATTCAATCTTACT                                     | Detection exterior of <i>perR</i> mutant.                           |
| perR-interior-F/R      | TTAGTTCTGGCAATCAGGACAAATG<br>GACCACCCTAGTGCTGAAATGA   | Detection interior of <i>perR</i> mutant.                           |
| perR-complementary-F/R | AAAAC TGCAGCACAGGAAAAGTATGTAAACAAGTC<br>CGCGGATCCTTAGTTCTGGCAATCAGGACAAATG                  | Amplification of <i>perR</i> for constructing the complement strain |

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Supplementary Table 3

| Gene ID         | log2FoldChange | gene_description   |
|-----------------|----------------|--|
| SSUSC84_RS09770 | -4.48845       | metal ABC transporter permease && PF00950:ABC 3 transport family                         |
| SSUSC84_RS09780 | -2.6577        | metal ABC transporter ATP-binding protein && PF00005:ABC transporter                     |
| SSUSC84_RS09775 | -3.72624       | metal ABC transporter permease && PF00950:ABC 3 transport family                         |
| SSUSC84_RS09790 | -1.96471       | zinc ABC transporter substrate-binding protein && PF01297:Zinc-uptake complex component  |
| SSUSC84_RS09765 | -1.32004       | A periplasmic  |
| SSUSC84_RS01570 | -1.58407       | metalloendopeptidase && PF05649:Peptidase family M13 PF01431:Peptidase family M13        |
| Novel00011      | -2.05535       | heavy metal translocating P-type ATPase && PF00122:E1-E2 ATPase PF00702:haloacid         |
| SSUSC84_RS02040 | -0.51407       | dehalogenase-like hydrolase  |
| SSUSC84_RS02105 | -0.56603       | -  |
| SSUSC84_RS03075 | 1.27275        | glucosamine-6-phosphate deaminase && PF01182:Glucosamine-6-phosphate                     |
| SSUSC84_RS08530 | 0.777048       | isomerases/6-phosphogluconolactonase   |
| SSUSC84_RS01435 | 0.931598       | ribonuclease && -  |
| SSUSC84_RS07830 | 0.716752       | bifunctional acetaldehyde-CoA/alcohol dehydrogenase && PF00171:Aldehyde dehydrogenase    |
| SSUSC84_RS09870 | 0.967297       | family PF00465:Iron-containing alcohol dehydrogenase                                     |
| SSUSC84_RS07840 | 0.864321       | VWA domain-containing protein && PF00092:von Willebrand factor type A domain             |
| SSUSC84_RS00470 | 0.586637       | discoidin domain-containing protein && PF00754:F5/8 type C domain PF05738:Cna protein    |
| SSUSC84_RS09875 | 0.938745       | B-type domain  |
| SSUSC84_RS06470 | 0.567868       | YSIRK-type signal peptide-containing protein && -  |
| SSUSC84_RS01400 | 0.562042       | 50S ribosomal protein L16 && PF00252:Ribosomal protein L16p/L10e                         |
| SSUSC84_RS09860 | 0.919182       | carboxypeptidase regulatory-like domain-containing protein && PF05738:Cna protein B-type |
| SSUSC84_RS03030 | 1.025474       | domain   |
| SSUSC84_RS00580 | 0.575282       | LPXTG cell wall anchor domain-containing protein && PF03377:Xanthomonas avirulence       |

