

1

2 Fig S1 Impact of *rafX* gene deletion on WTA-PG banding pattern in the genetic backgrounds of TIGR4
 3 (Serotype 4), CMCC (B)31203 (Serotype 3) and *S. mitis* W1. Pneumococcal cells were then lysed by
 4 incubating the cultures at 37 °C for 30 min in the presence of 0.4% sodium deoxycholate; Whereas *S. mitis*
 5 cells were digested with 300 U/ml mutanolysin (Sigma-Aldrich) and 5 mg/ml lysozyme (Sigma-Aldrich) in
 6 50 mM Tris buffer (pH 7.5) at 37 °C for 4 hours. TA samples were separated on 10% SDS-PAGE gels and
 7 immunoblotted with monoclonal mouse anti-*P*-Cho IgA (TEPC-15). 1, 2 and 3 denote wild type strain,
 8 Δ *rafX* mutant, and complemented strain, respectively. 3* denote R6 Δ *rafX* mutant was ectopically
 9 complemented with SM12261_0467 from strain *S. mitis* NCTC12261. The *S. mitis* W1 Δ *smi0513* mutant
 10 was constructed by insertion-duplication mutagenesis as described elsewhere (1). Briefly, the internal *rafX*
 11 fragment was PCR amplified with primers Wuk13 and Wuk14. The resulting DNA fragment was cloned into
 12 plasmid pEVP3 after enzyme digestion to generate plasmid pYYB13. This plasmid was then used to
 13 transform *S. mitis* W1 following protocols to transform *S. pneumoniae* (2).

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Contig_ST320_I 1050
Contig_ST2296_I 1050
Contig_ST242_I 1050
Contig_ST876_I 1050
Contig_ST910_I 1050
Contig_ST81_I 1050
Contig_ST2754_I 1050
Contig_ST320_I 1050
Contig_ST902_I 1050
Contig_ST910_I 1050
Contig_ST271_I 1050
Contig_ST271_I 1050
Contig_ST4112_I 1050
Contig_ST3173_I 1050
Contig_ST3263_I 1050
Contig_D39 1050
Contig_ST271_C 1050
Contig_ST910_C 1050
Contig_ST280_C 1050
Contig_ST338_C 1050
Contig_ST910_C 1050
Contig_ST3173_C 1050
Contig_ST8781_C 1050
Contig_ST2754_C 1050
Contig_ST910_C 1050
Contig_ST242_C 1050
Contig_ST320_C 1050
Contig_ST6791_C 1050
Contig_ST4745_C 1050
Contig_ST4113_C 1050
Contig_ST876_C 1050
Contig_TIGR_4 1050
Consensus 1050

Contig_ST320_I 1200
Contig_ST2296_I 1200
Contig_ST242_I 1200
Contig_ST876_I 1200
Contig_ST910_I 1200
Contig_ST81_I 1200
Contig_ST2754_I 1200
Contig_ST320_I 1200
Contig_ST902_I 1200
Contig_ST910_I 1200
Contig_ST271_I 1200
Contig_ST271_I 1200
Contig_ST4112_I 1200
Contig_ST3173_I 1200
Contig_ST3263_I 1200
Contig_D39 1200
Contig_ST271_C 1200
Contig_ST910_C 1200
Contig_ST280_C 1200
Contig_ST338_C 1200
Contig_ST910_C 1200
Contig_ST3173_C 1200
Contig_ST8781_C 1200
Contig_ST2754_C 1200
Contig_ST910_C 1200
Contig_ST242_C 1200
Contig_ST320_C 1200
Contig_ST6791_C 1200
Contig_ST4745_C 1200
Contig_ST4113_C 1200
Contig_ST876_C 1200
Contig_TIGR_4 1200
Consensus 1200

Contig_ST320_I 1311
Contig_ST2296_I 1311
Contig_ST242_I 1311
Contig_ST876_I 1311
Contig_ST910_I 1311
Contig_ST81_I 1311
Contig_ST2754_I 1311
Contig_ST320_I 1311
Contig_ST902_I 1311
Contig_ST910_I 1311
Contig_ST271_I 1311
Contig_ST271_I 1311
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Contig_ST6791_C 1311
Contig_ST4745_C 1311
Contig_ST4113_C 1311
Contig_ST876_C 1311
Contig_TIGR_4 1311
Consensus 1311

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18 Fig S2 Conservation of *rafX* gene among clinical isolated strains by sequencing. The underlines indicate the
19 initial codon of TTG and the terminal codon of TAA.

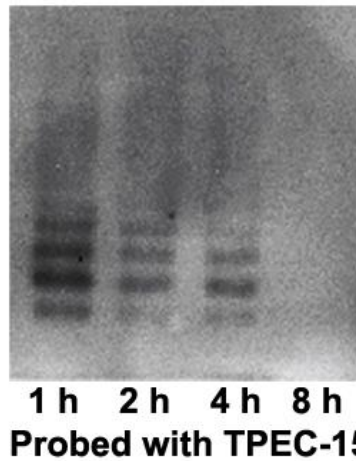


Fig S3 Banding pattern of WTA-PG from R6 $\Delta rafX$ mutant following digestion with enzymes. The enzymes (mutanolysin plus lysozyme) digested R6 $\Delta rafX$ cells were sedimented after a 2-h digestion and aliquots of the supernatants were incubated at 37 °C with shaking for another 1, 2, 4 and 8 h. TA samples were separated on 10% SDS-PAGE gels and immunoblotted with TEPC-15.

Download Graphics Sort by: E value

gi|15600192|ref|NP_253686.1| O-antigen ligase, WaaL [Pseudomonas aeruginosa PAO1]
Sequence ID: lcl|10625 Length: 401 Number of Matches: 2

Range 1: 264 to 322 Graphics Next Match Previous Match

| Score | Expect | Method | Identities | Positives | Gaps |
|-------------------|--------|--|------------|------------|-----------|
| 18.9 bits(37) | 0.28 | Compositional matrix adjust. | 15/63(24%) | 29/63(46%) | 7/63(11%) |
| RafX Query | 266 | RISINDAGMALFKQNPFWGEG---FLTYMHSYPRIHAPYHEHANSLYIDTILSYGIVGTI | 306 | 322 | |
| | | R IW + ++P+ G G P+ + S + A H++ + ++ GI+G + | | | |
| WaaL Sbjct | 264 | RPEIWADALRQISEHPWLGHGVDHPMRIVLSNGMLLA----DPHNIELGVLFAGGIIGLL | 303 | 319 | |
| Query | 323 | LLV 325 | | | |
| | | L V | | | |
| Sbjct | 320 | LWV 322 | | | |

Fig S4 Alignment of *S. pneumoniae* RafX with *P. aeruginosa* PAO1 WaaL using BLAST database. RafX contains a H306 residue which is structurally equivalent to the H303 residue of *P. aeruginosa* PAO1 WaaL.

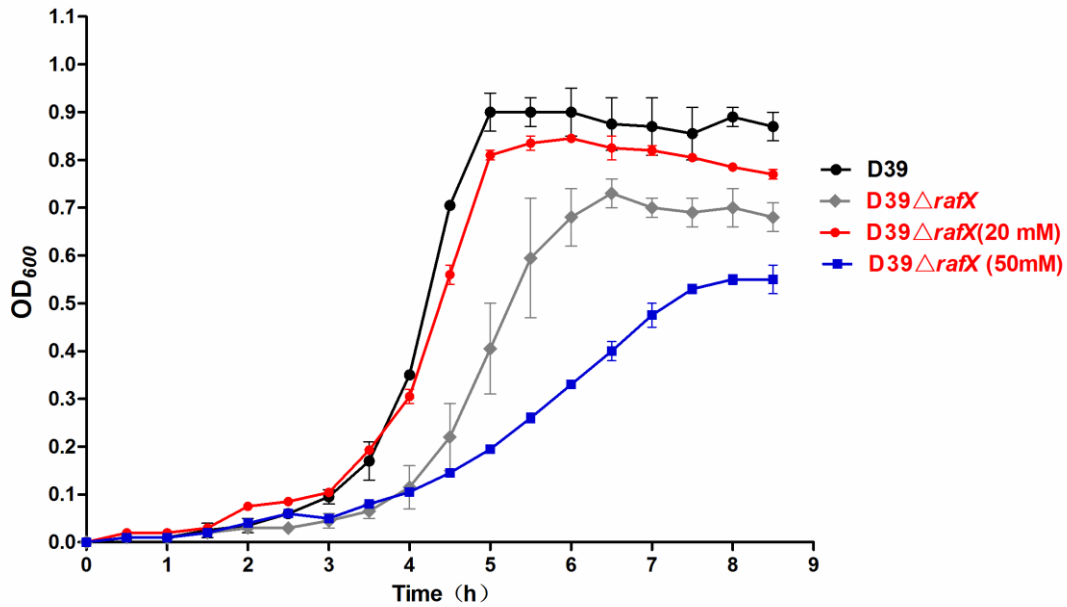


FIG S5 Growth curve of bacteria grown in the indicated media. Bacteria were grown in C+Y medium at 37 °C, and media for D39 Δ rafX mutant were supplemented with the indicated concentrations of MgCl₂. Absorbance measurements were taken every 1 h using a 722s Spectrophotometer.

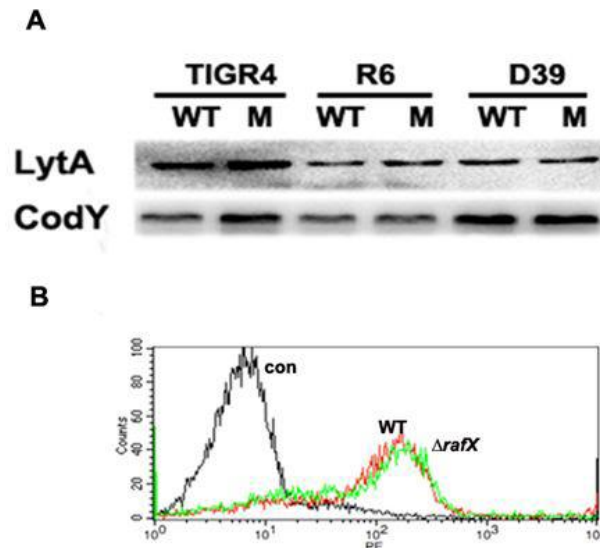


FIG S6 LytA expression was not influenced by *rafX* deletion. (A) Pneumococcal bacteria harvested at mid-exponential phase, and the total amounts of LytA in bacterial lysates of pneumococcal strains were determined by Western blotting with polyclonal mouse anti-LytA serum. M denotes Δ rafX. (B) Fluorescence-activated cell sorting analysis was performed to show surface-exposed LytA in the wild type R6 strain and the Δ rafX mutant. Bacterial cells were treated with mouse antisera against LytA recombinant protein (anti-LytA antibody, dilution 1: 100) in blocking buffer (5% fetal bovine serum, 1 \times PBS) for 60 min at 37 °C. Con indicate that R6 strain was incubated with normal mouse serum. The bacterial pellets were washed three times with PBS and incubated with goat anti-mouse IgG-PE secondary antibodies at 1: 200 (Santa Cruz Biotech) for 60 min at 4 °C.

47 **REFERENCES**

- 48 1. **Zhang J-R, Idanpaan-Heikkila I, Fischer W, Tuomanen E.** 1999. Pneumococcal *licD2* gene is
49 involved in phosphorylcholine metabolism. *Mol Microbiol.* **31**:1477-1481.
- 50 2. **Wu K, Zhang X, Shi J, Li N, Li D, Luo M, Cao J, Yin N, Wang H, Xu W, He Y, Yin Y.** 2010.
51 Immunization with a combination of three pneumococcal proteins confers additive and broad
52 protection against *Streptococcus pneumoniae* infections in mice. *Infect Immun.* **78**:1276-1283.