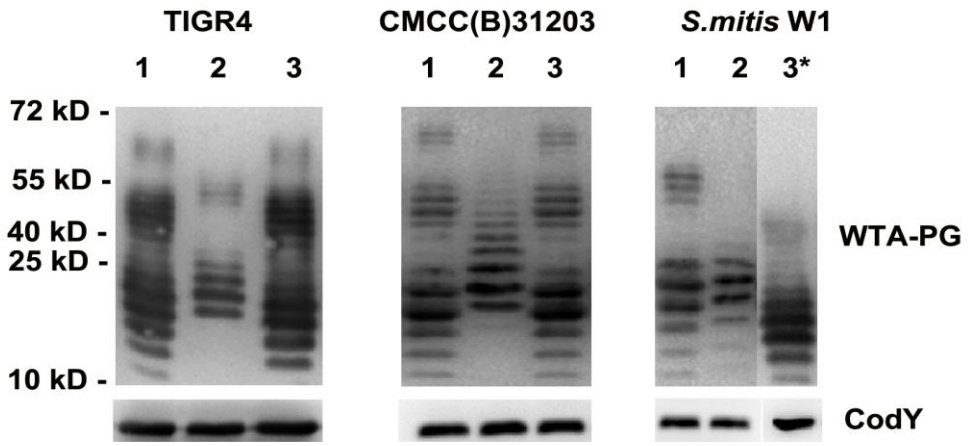
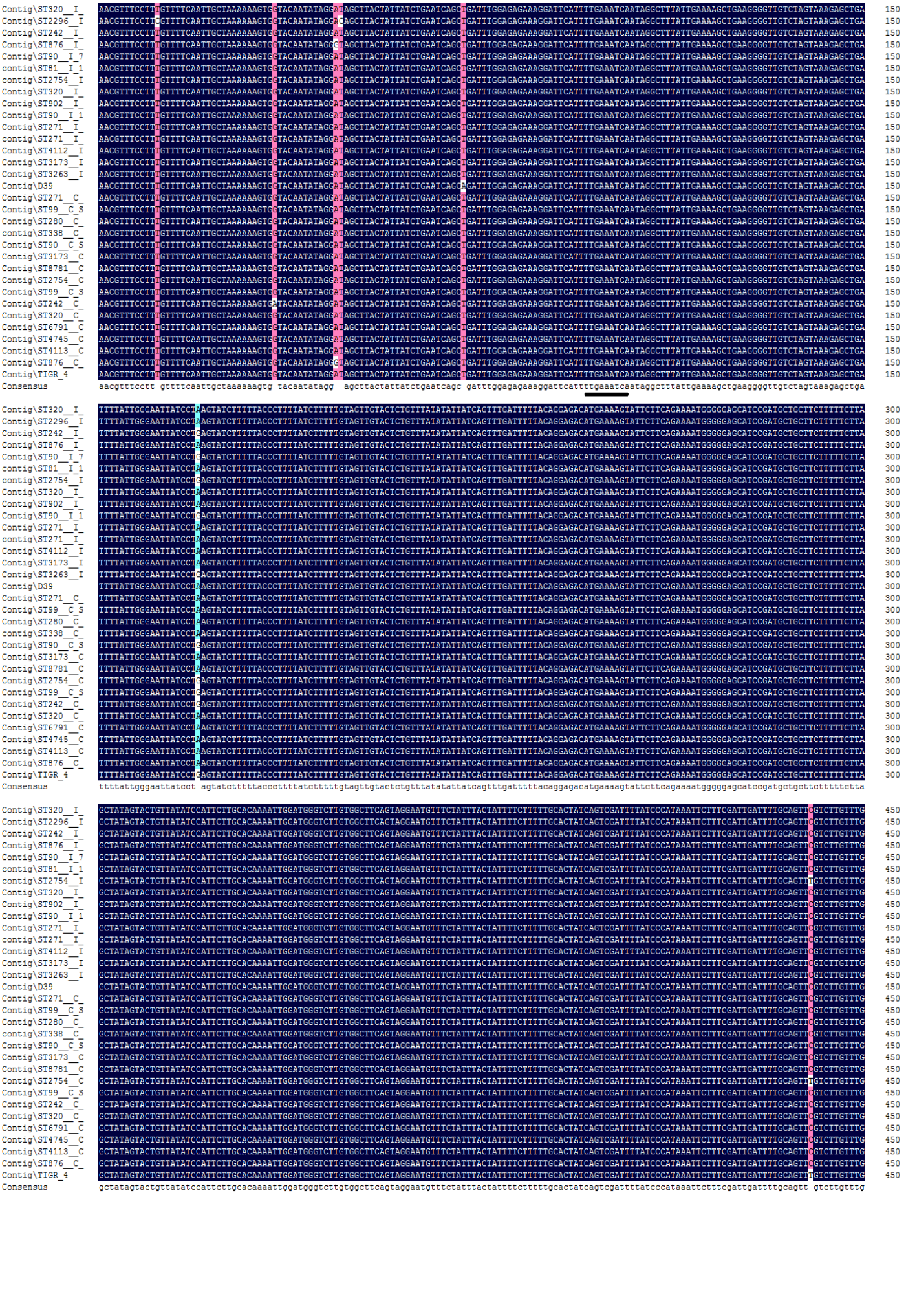


Fig S1 Impact of *rafX* gene deletion on WTA-PG banding pattern in the genetic backgrounds of TIGR4 (Serotype 4), CMCC (B)31203 (Serotype 3) and *S. mitis* W1. Pneumococcal cells were then lysed by incubating the cultures at 37 ℃ for 30 min in the presence of 0.4% sodium deoxycholate; Whereas *S. mitis* cells were digested with 300 U/ml mutanolysin (Sigma-Aldrich) and 5 mg/ml lysozyme (Sigma-Aldrich) in 50 mM Tris buffer (pH 7.5) at 37 ℃ for 4 hours. TA samples were separated on 10% SDS-PAGE gels and immunoblotted with monoclonal mouse anti-*P*-Cho IgA (TEPC-15). 1, 2 and 3 denote wild type strain, Δ*rafX* mutant, and complemented strain, respectively. 3* denote R6 Δ*rafX* mutant was ectopically complemented with SM12261_0467 from strain *S. mitis* NCTC12261. The *S. mitis* W1 Δ*smi0513* mutant was constructed by insertion-duplication mutagenesis as described elsewhere (1). Briefly, the internal *rafX* fragment was PCR amplified with primers Wuk13 and Wuk14. The resulting DNA fragment was cloned into plasmid pEVP3 after enzyme digestion to generate plasmid pYYB13. This plasmid was then used to transform *S. mitis* W1 following protocols to transform *S. pneumoniae* (2).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Contig\ST2296__I
Contig\ST242__I_
Contig\ST876__I_
Contig\ST90__I_7
Contig\ST81__I_1
Contig\ST2754__I
Contig\ST320__I_
Contig\ST902__I_
Contig\ST90__I_1
Contig\ST271__I_
Contig\ST271__I_
Contig\ST4112__I
Contig\ST3173__I
Contig\ST3263__I
Contig\D39
Contig\ST271__C_
Contig\ST99__C_S
Contig\ST280__C_
Contig\ST338__C_
Contig\ST90__C_S
Contig\ST3173__C
Contig\ST8781__C
Contig\ST2754__C
Contig\ST99__C_S
Contig\ST242__C_
Contig\ST320__C_
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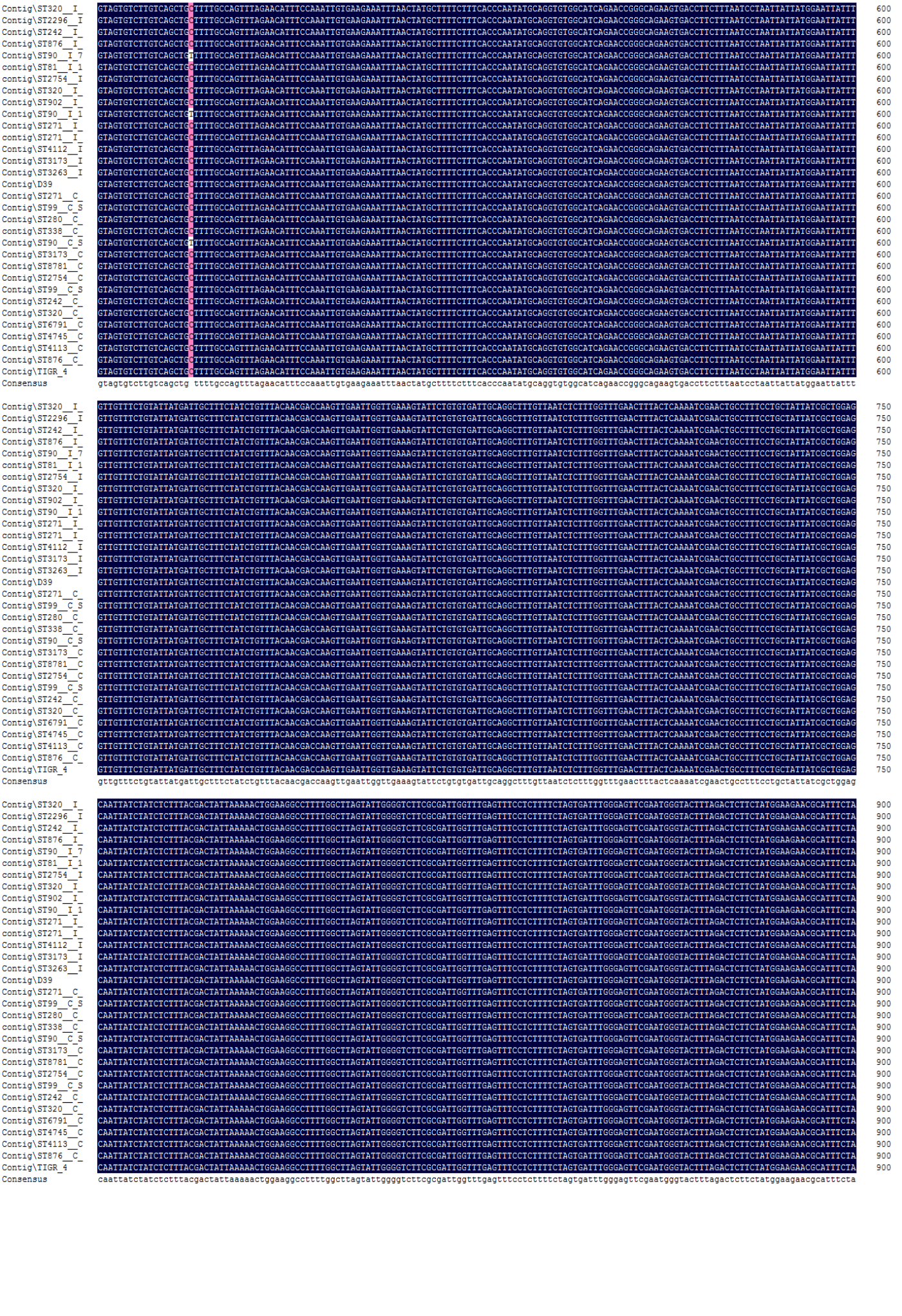
Contig\TIGR_4
Consensus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Contig\ST4112__I
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Contig\ST99__C_S
Contig\ST280__C_
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Contig\ST99__C_S
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Consensus
16

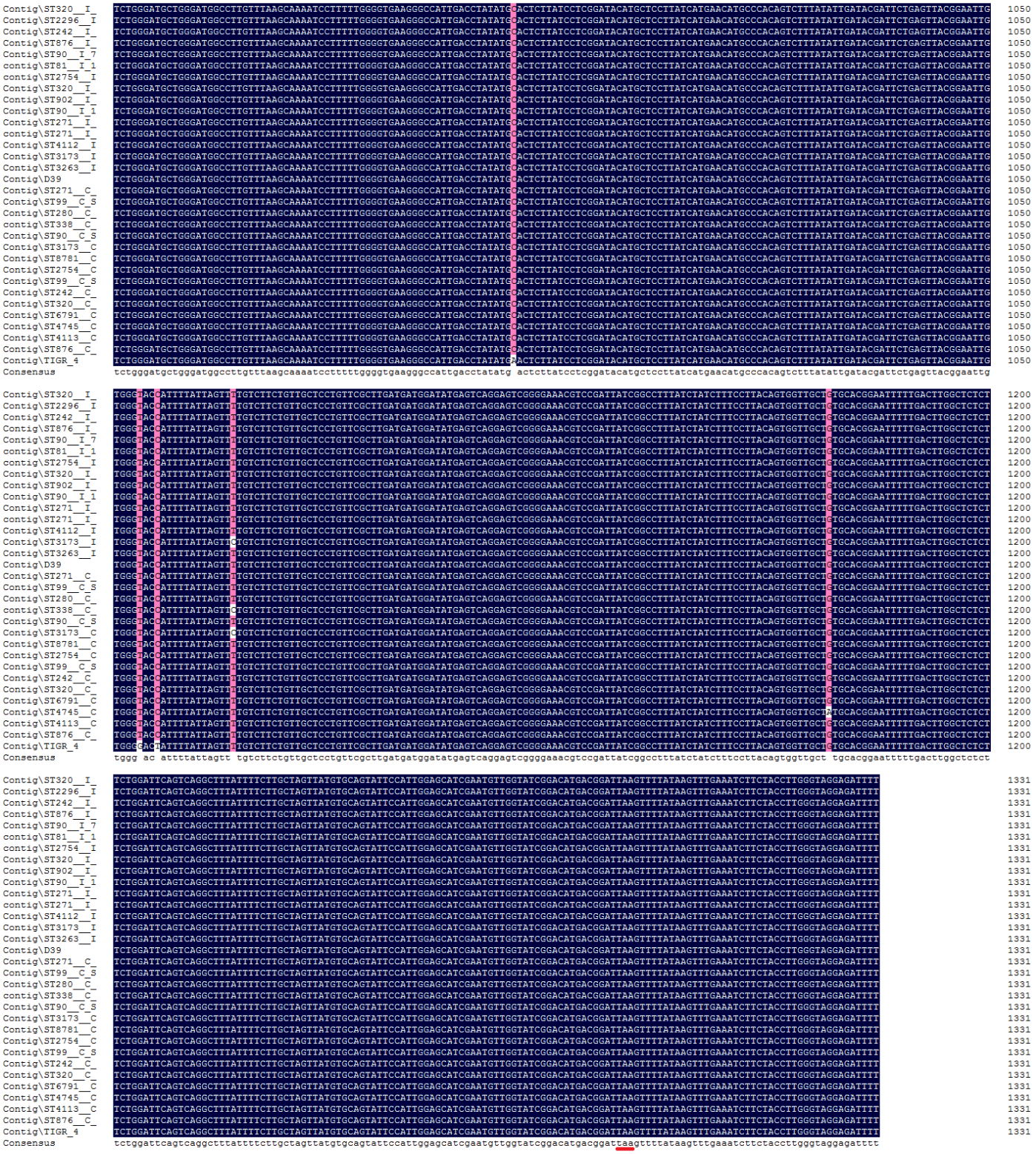

Fig S2 Conservation of *rafX* gene among clinical isolated strains by sequencing. The underlines indicate the initial codon of TTG and the terminal codon of TAA.

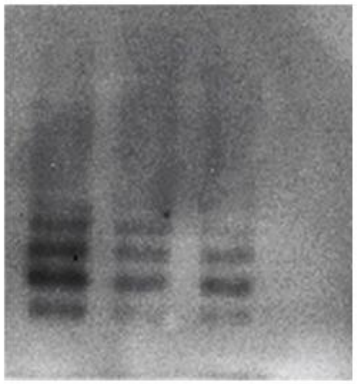

Fig S3 Banding pattern of WTA-PG from R6 Δ*rafX* mutant following digestion with enzymes. The enzymes (mutanolysin plus lysozyme) digested R6 Δ*rafX* cells were sedimented after a 2-h digestion and aliquots of the supernatants were incubated at 37 ℃ with shaking for another 1, 2, 4 and 8 h. TA samples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

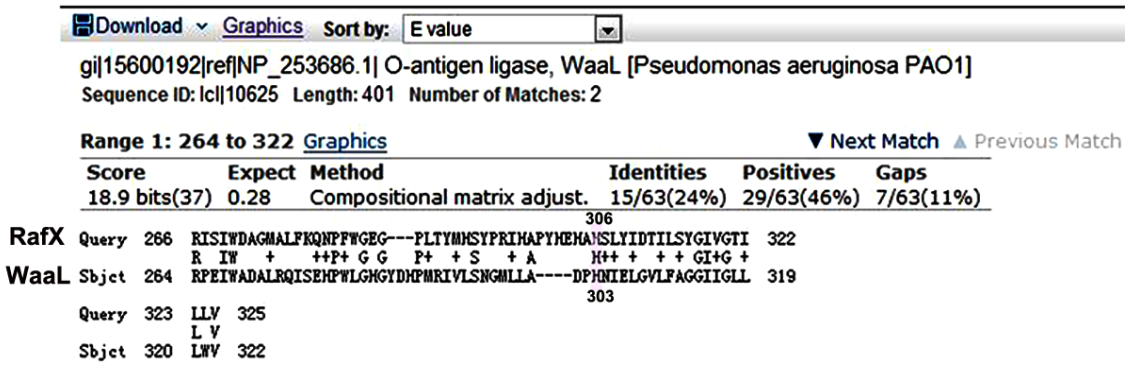

Fig S4 Alignment of *S. pneumoniae* RafX with *P. aeruginosa* PAO1 WaaL using BLAST database. RafX contains a H306 residue which is structurally equivalent to the H303 residue of *P. aeruginosa* PAO1 WaaL.

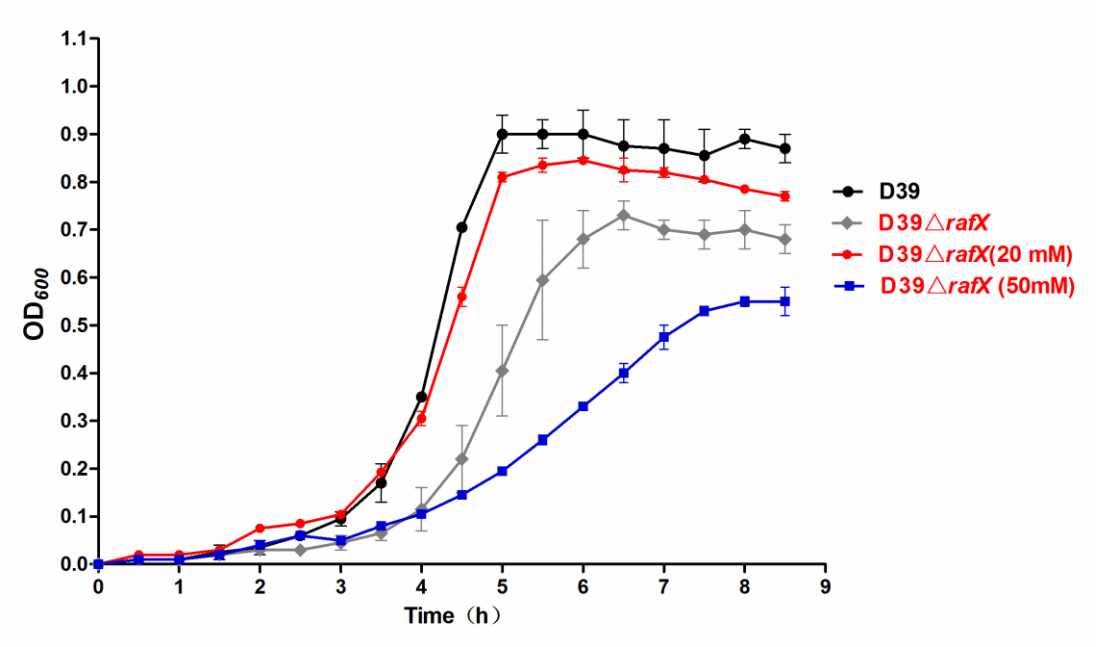

FIG S5 Growth curve of bacteria grown in the indicated media. Bacteria were grown in C+Y medium at 37 ℃, and media for D39 Δ*rafX* mutant were supplemented with the indicated concentrations of $MgCl_2$. Absorbance measurements were taken every 1 h using a 722s Spectrophotometer.

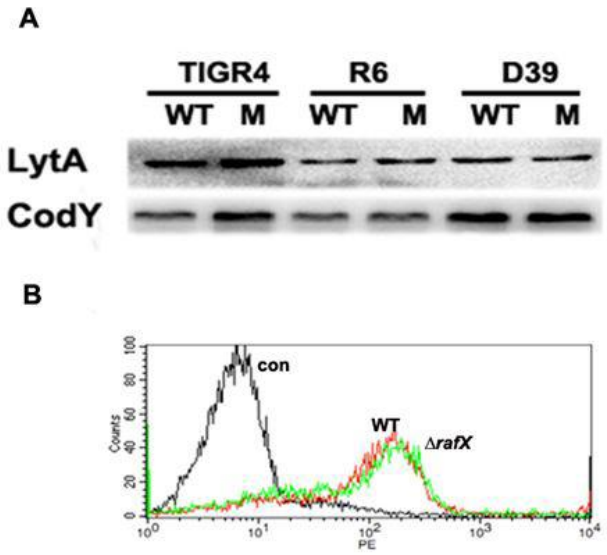

FIG S6 LytA expression was not influenced by *rafX* deletion. **(**A) Pneumococcal bacteria harvested at mid-exponential phase, and the total amounts of LytA in bacterial lysates of pneumococcal strains were determined by Western blotting with polyclonal mouse anti-LytA serum. M denotes Δ*rafX*. (B) 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% fatal bovine serum, 1 ×PBS) for 60 min at 37 ℃. 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 ℃.

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1. **Zhang J-R, Idanpaan-Heikkila I, Fischer W, Tuomanen E.** 1999. Pneumococcal *licD2* gene is involved in phosphorylcholine metabolism. Mol Microbiol. **31:**1477-1481.
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. Immunization with a combination of three pneumococcal proteins confers additive and broad protection against *Streptococcus pneumoniae* infections in mice. Infect Immun. **78:**1276-1283.