

Fig S1 Impact of *rafX* gene deletion on WTA-PG banding pattern in the genetic backgrounds of TIGR4 2 (Serotype 4), CMCC (B)31203 (Serotype 3) and S. mitis W1. Pneumococcal cells were then lysed by 3 incubating the cultures at 37 °C for 30 min in the presence of 0.4% sodium deoxycholate; Whereas S. mitis 4 cells were digested with 300 U/ml mutanolysin (Sigma-Aldrich) and 5 mg/ml lysozyme (Sigma-Aldrich) in 5 50 mM Tris buffer (pH 7.5) at 37 °C for 4 hours. TA samples were separated on 10% SDS-PAGE gels and 6 immunoblotted with monoclonal mouse anti-P-Cho IgA (TEPC-15). 1, 2 and 3 denote wild type strain, 7  $\Delta rafX$  mutant, and complemented strain, respectively. 3\* denote R6  $\Delta rafX$  mutant was ectopically 8 complemented with SM12261\_0467 from strain S. mitis NCTC12261. The S. mitis W1 Asmi0513 mutant 9 was constructed by insertion-duplication mutagenesis as described elsewhere (1). Briefly, the internal rafX10 fragment was PCR amplified with primers Wuk13 and Wuk14. The resulting DNA fragment was cloned into 11 plasmid pEVP3 after enzyme digestion to generate plasmid pYYB13. This plasmid was then used to 12 13 transform S. mitis W1 following protocols to transform S. pneumoniae (2).

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Contig\ST320_I_	AACGTTTCCTT <mark>T</mark> GTTTTC7	ATTGCTAAAAAAGTC <mark>G</mark>	TACAATATAGG <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	T <mark>GATTTGGAGAGAAAGGATTCATTT</mark>	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST2296_I	AACGTTTCCTTCGTTTTC/	ATTGCTAAAAAAGTGG	TACAATATAGGACAGCTT	ACTATTATCTGAATCAG	GATTTGGAGAGAAAGGATTCATTT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST242_I_	AACGTTTCCTTTGTTTTC2	ATTGCTAAAAAAGTG	TACAATATAGCATAGCTT	ACTATTATCTGAATCAG	GATTTGGAGAGAAAGGATTCATTI	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST876_I_	AACGTTTCCTTTGTTTTC	ATTGCTAAAAAAGTGG	TACAATATAGeGTAGCTT	ACTATTATCTGAATCAG	IGATTTGGAGAGAAAGGATTCATTT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST90_1_7	AACGTTTCCTTTGTTTTC	ATTGCTAAAAAAGTGG	TACAATATAGCATAGCTT	ACTATTATCTGAATCAG	GATTTGGAGAGAAAGGATTCATTT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
contig\ST81_1_1	AACGTTTCCTTTGTTTTCA	ATTGCTAAAAAAGTGG	TACAATATAGCATAGCTT	ACTATTATCTGAATCAG	GATTIGGAGAGAAAGGATICATII	TGAAATCAATAGGCTTTATTGAAAAG	CIGAAGGGGTIGICIAGIAAAG	AGCTGA 15
Contig(512/54_1	AACGITICCTTIGITITCA		TACAATATAGGATAGCII	ACTATIAICIGAAICAG		TGAAAICAAIAGGCIIIAIIGAAAAG	CIGAAGGGGIIGICIAGIAAAG	AGCIGA 15
Contig(SI320_1_	AACGITICCTT	ATTCCTANAAAAGIGG	TACAATATAGGATAGCII	ACTATIAICIGAAICAG		TCAAAICAAIAGGCIIIAIIGAAAAG	CIGAAGGGGIIGICIAGIAAAG	AGCIGA 15
Contig(SI902_1_	AACGITICCITIGITICA	ATTCCTANANAGIGG	TACAMINIAGCAIAGCII	ACTATIAICIGAAICAG	CATTICCACACACAACCATTCATT	TCANATCANTAGGCITIATIGAAAAG	CTCAAGGGGIIGICIAGIAAAG	AGCIGA 15
Contig(SISO_I_I	AACCTTTCCTTTCTTTC	ATTCCTANANAGIGG	TACAMININGCAINGCII	ACTATINICIGANICAG		TCANATCANTAGGCITIATIGAAAAC	CTCAACGGGTTGTCTAGTAAAG	AGCIGA 15
contig\ST271_I	AACGTTTCCTTTCTTTCZ	ATTCCTAAAAAGIGG	TACAATATAGGATAGCII	ACTATTATCTCAATCAG	CATTICGAGAGAGAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAC	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig(ST271_1_	AACGTTTCCTTTGTTTTCA	ATTECTAAAAAAGTGG	TACAATATAGGATAGCII	ACTATTATCTGAATCAG	GATTTGGAGAGAAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST3173 I	AACGTTTCCTTTGTTTTC	ATTGCTAAAAAAGTGG	TACAATATAGGATAGCII	ACTATTATCTGAATCAG	TGATTTGGAGAGAGAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST3263 I	AACGTTTCCTTTGTTTTC	ATTGCTAAAAAAGTG	TACAATATAGGATAGCTT	ACTATTATCTGAATCAG	TGATTTGGAGAGAAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAA	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\D39	AACGTTTCCTTTGTTTTC	ATTGCTAAAAAAGTG	TACAATATAGCATAGCTT	ACTATTATCTGAATCAG	GATTTGGAGAGAAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST271 C	AACGTTTCCTTTGTTTTCZ	ATTGCTAAAAAAGTG	TACAATATAGG <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	TGATTTGGAGAGAAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST99 C S	AACGTTTCCTT <mark>T</mark> GTTTTC2	ATTGCTAAAAAAGTG	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	C <mark>T</mark> GATTTGGAGAGAAAGGATTCATTI	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST280 C	AACGTTTCCTTTGTTTTC	ATTGCTAAAAAAGTG	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	TGATTTGGAGAGAAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
contig\ST338 C	AACGTTTCCTT <mark>T</mark> GTTTTC	ATTGCTAAAAAAGTG	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	TGATTTGGAGAGAAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST90 C S	AACGTTTCCTT <mark>T</mark> GTTTTC	ATTGCTAAAAAAGTG	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	TGATTTGGAGAGAAAGGATTCATT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST3173 C	AACGTTTCCTT <mark>T</mark> GTTTTC <i>I</i>	ATTGCTAAAAAAGTG <mark>G</mark>	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	• <mark>T</mark> GATTTGGAGAGAAAGGATTCATT1	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST8781_C	AACGTTTCCTT <mark>T</mark> GTTTTC <i>I</i>	ATTGCTAAAAAAGTC <mark>G</mark>	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	C <mark>T</mark> GATTTGGAGAGAAAGGATTCATT1	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST2754_C	AACGTTTCCTT <mark>T</mark> GTTTTC <i>I</i>	ATTGCTAAAAAAGTC <mark>G</mark>	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	O <mark>T</mark> GATTTGGAGAGAAAGGATTCATTI	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST99_C_S	AACGTTTCCTT <mark>T</mark> GTTTTC <i>I</i>	ATTGCTAAAAAAGTG <mark>G</mark>	ТАСААТАТАСС <mark>АТ</mark> АССТТ	ACTATTATCTGAATCAG	TGATTTGGAGAGAAAGGATTCATTI	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST242_C_	AACGTTTCCTT <mark>T</mark> GTTTTC7	ATTGCTAAAAAAGTG <mark>A</mark>	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	T <mark>GATTTGGAGAGAAAGGATTCATTT</mark>	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST320_C_	AACGTTTCCTT <mark>T</mark> GTTTTC/	ATTGCTAAAAAAGTG <mark>G</mark>	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	TGATTTGGAGAGAAAGGATTCATTT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST6791_C	AACGTTTCCTT <mark>T</mark> GTTTTC <i>I</i>	ATTGCTAAAAAAGTG <mark>G</mark>	TACAATATAGC <mark>AT</mark> AGCTT	ACTATTATCTGAATCAG	C <mark>T</mark> GATTTGGAGAGAAAGGATTCATTI	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST4745_C	AACGTTTCCTTTGTTTTC2	ATTGCTAAAAAAGTG	TACAATATAGCATAGCTT	ACTATTATCTGAATCAG	GATTTGGAGAGAAAGGATTCATTI	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig\ST4113_C	AACGTTTCCTTTGTTTTC/	ATTGCTAAAAAAGTG	TACAATATAGCATAGCTT	ACTATTATCTGAATCAG	GATTTGGAGAGAAAGGATTCATTT	TGAAATCAATAGGCTTTATTGAAAAG	CTGAAGGGGTTGTCTAGTAAAG	AGCTGA 15
Contig(SI8/6_C_	AACGITICCTTTGTTTTCA		TACAATATAGGGTAGCII	ACTATIAICIGAAICAG		IGAAAICAAIAGGUIIIAIIGAAAAG	CIGAAGGGGIIGICIAGIAAAG	AGCIGA 15
Contig (IIGR_4				ACTATIAICIGAAICAG		IGAAAICAAIAGGCIIIAIIGAAAAG	CIGAAGGGGIIGICIAGIAAAG	AGCIGA 15
Consensus	aacgtttcctt gttttca	attgctaaaaagtg	tacaatatagg agett	actattatctgaatcag	c gatttggagagaaaggattcattt	rgaaatcaataggetttattgaaaag	ctgaaggggttgtctagtaaag	agetga
Contig ST220 T	TTTTATTCCCAATTATCC	ACTATCTTTTACCC	TTTTATCTTTTTCTACTT	CTACTCTCTTTATATAT	TATCACTTTCATTTTACACCACACA	TCAAAACTATTCTTCACAAAATCCCC	CACCATCCCATCCTCCTTCTTT	TTCTT3 20
Contig(ST320_1_	TTTTATTGGGAATTATCC	AGIAICIIIIIACCC	TTTTATCTTTTTCTACT	GINCICIGIIIMIMIMI	TATCAGITIGATITITACAGGAGACA	TGANANGTATICTICAGANANTGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig(ST2230_1	TTTTATTGGGAATTATCC	GAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCIGITIATATATA GTACTCTGTTTATATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST876 I	TTTTATTGGGAATTATCC	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST90 I 7	TTTTATTGGGAATTATCC	GAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
contig\ST81 I 1	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
contig\ST2754 I	TTTTATTGGGAATTATCC	GAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST320 I	TTTTATTGGGAATTATCC	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST902 I	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST90 I 1	TTTTATTGGGAATTATCCI	GAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST271 I	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
contig\ST271_I	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST4112_I	TTTTATTGGGAATTATCC	AAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST3173_I	TTTTATTGGGAATTATCC	A AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST3263_I	TTTTATTGGGAATTATCCI	GAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\D39	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST271_C_	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST99_C_S	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST280_C_	TTTTATTGGGAATTATCC	AAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
contig\ST338_C_	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST90_C_S	TTTTATTGGGAATTATCCI	GAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig(SI31/3_C	TITIATIGGGAATTATCC	AAGIAICIIIIIACCC	TITIAICITITIGIAGII	GIACICIGIIIAIAIAI			GAGCAICCGAIGCIGCIICIII	TICITA 30
Contig(518/81_C	TTTTATIGGGAATTATCC	AAGIAICIIIIIACCC	TTTTATCITITIGIAGII	GIACICIGIIIAIAIAI CTA CTOTOTOTTTATATAT			GAGCATCCCGATGCTGCTTCTTT	TICITA 30
Contig(SI2/54_C	TTTTATTGGGAATTATCC	CAGINICITITIACCC	TTTTAICIIIIIGIAGII	GIACICIGIIIAIAIAI GTACTCTCTTTATATAT	TATCAGITIGATITITACAGGAGACA	TCARAAAGIAIICIICAGAAAAIGGGG	GAGCATCCGATGCIGCIICIII	TTCTTA 30
Contig(ST242 C	TTTTATTGGGAATTATCC	GAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTCTCTTTATATAT	TATCAGTTTGATTTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig(ST212_C_	TTTTATTGGGAATTATCC	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTTACAGGAGAGA	TGAAAAGTATTCTTCAGAAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST6791 C	TTTTATTGGGAATTATCC	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST4745 C	TTTTATTGGGAATTATCC	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST4113 C	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\ST876 C	TTTTATTGGGAATTATCCI	AGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Contig\TIGR 4	TTTTATTGGGAATTATCC	GAGTATCTTTTTACCC	TTTTATCTTTTTGTAGTT	GTACTCTGTTTATATAT	TATCAGTTTGATTTTTACAGGAGACA	TGAAAAGTATTCTTCAGAAAATGGGG	GAGCATCCGATGCTGCTTCTTT	TTCTTA 30
Consensus	ttttattgggaattatcct	agtatctttttaccc	ttttatctttttgtagtt	gtactctgtttatatat	tatcagtttgatttttacaggagaca	tgaaaagtattetteagaaaatgggg	gagcatccgatgctgcttcttt	ttetta
Contig\ST320_I_	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45
Contig\ST2296_I	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45
Contig\ST242_I_	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45
Contig\ST876_I_	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTI	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	ngnnng 45
Contig\ST90_1_7	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	ICAGTAGGAATGTTTCT	ATTTACTATTTTTTTTTTTGCACTATC	AGICGATITTATCCCATAAATTCTTT	CGATTGATTTTGCAGTTCGTCT	IGITIG 45
contig\ST81_1_1	GCTATAGTACTGTTATATO	CATICITGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT		AGTCGATITIATCCCATAAATICTTI	CGATTGATTTTGCAGTTGGTCT	TGITIG 45
Contig(SI2/54_1	CCTATAGIACIGITATAT	CATICIIGCACAAAAI	TCCATCCCTCTTCTCCCCT	TCAGIAGGAAIGIIICI.	ATTACTATITICTITIGCACIAC	AGICGATITIAICCCATAAATICITI	CCATTCATTTTCCAGITIGICI	TCTTTC 45
Contig(SIS20_1_	CCTATAGIACIGIIAIAI	CATTCTTCCACAAAAA	TECATEGETCTTCTCCCT	TCAGINGGAMIGITICI.	ATTTACTATTTTCTTTTTCCACTATC	AGICGATITIAICCCATAAATICIII	CGATIGATITICCAGITCGICI	TETTTE 45
Contig\ST90 T 1	GCTATAGTACTGTTATAT	CATTCTTGCACAAAAI	TGGATGGGTCTTCTGCCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTCCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTTCGTCT	TGTTTG 45
Contig\ST271 T	GCTATAGTACTGTTATAT	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTCCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTTCGTCT	TGTTTG 45
contig\ST271 I	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTTCGTCT	TGTTTG 45
Contig\ST4112 I	GCTATAGTACTGTTATAT	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTTCGTCT	TGTTTG 45
Contig\ST3173 I	GCTATAGTACTGTTATAT	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTTCGTCT	TGTTTG 45
Contig\ST3263 I	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45
Contig\D39	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTT1	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45
Contig\ST271_C	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45
Contig\ST99_C_S	GCTATAGTACTGTTATATO	CATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	CAGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45
Contig\ST280 C		CATTOTTCCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	ATTTACTATTTTCTTTTTGCACTATC	CAGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45
	GCTATAGTACTGTTATATO	CATICITICACACAAAAAI					CONTRONTTROCN CTT	
contig\ST338_C	GCTATAGTACTGTTATATO	CATICITGCACAAAAT	TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT	AIIIACIAIIIICIIIIIGCACIAIC	AGTCGATITTATCCCATAAATTCTTI	CGATIGATITIGCAGITC	TGTTTG 45
contig\ST338_C_ Contig\ST90_C_S	GCTATAGTACTGTTATATO GCTATAGTACTGTTATATO GCTATAGTACTGTTATATO	CCATTCTTGCACAAAAT CCATTCTTGCACAAAAT CCATTCTTGCACAAAAT	TGGATGGGTCTTGTGGCT TGGATGGGTCTTGTGGCT	TCAGTAGGAATGTTTCT. TCAGTAGGAATGTTTCT.	ATTTACTATTTTCTTTTTGCACTATC	AGTCGATTTTATCCCATAAATTCTTT AGTCGATTTTATCCCATAAATTCTTT	CGATTGATTTTGCAGTT <mark>C</mark> GTCT	TGTTTG 45 TGTTTG 45
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G	TAGTGTCTTGTC	AGCTCCTTTTG	CAGTTTAGAACAT	TTCCAAATTGTGA	AGAAATTTAACTA	TGCTTTTTCTTTCACCC	ATATGCAGGTGTGGG	ATCAGAACCGGG	CAGAAGTGACCT	ICTTTAATCCT	AATTATTATG	GAATTAT
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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.

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- 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.



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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  $\Delta rafX$  mutant were supplemented with the indicated concentrations of MgCl<sub>2</sub>. 34 Absorbance measurements were taken every 1 h using a 722s Spectrophotometer.



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FIG S6 LytA expression was not influenced by rafX deletion. (A) Pneumococcal bacteria harvested at 37 mid-exponential phase, and the total amounts of LytA in bacterial lysates of pneumococcal strains were 38 determined by Western blotting with polyclonal mouse anti-LytA serum. M denotes  $\Delta raf X$ . (B) 39 Fluorescence-activated cell sorting analysis was performed to show surface-exposed LytA in the wild type 40 R6 strain and the  $\Delta rafX$  mutant. Bacterial cells were treated with mouse antisera against LytA recombinant 41 protein (anti-LytA antibody, dilution 1: 100) in blocking buffer (5% fatal bovine serum, 1 × PBS) for 60 min 42 at 37 °C. Con indicate that R6 strain was incubated with normal mouse serum. The bacterial pellets were 43 washed three times with PBS and incubated with goat anti-mouse IgG-PE secondary antibodies at 1: 200 44 45 (Santa Cruz Biotech) for 60 min at 4 °C.

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