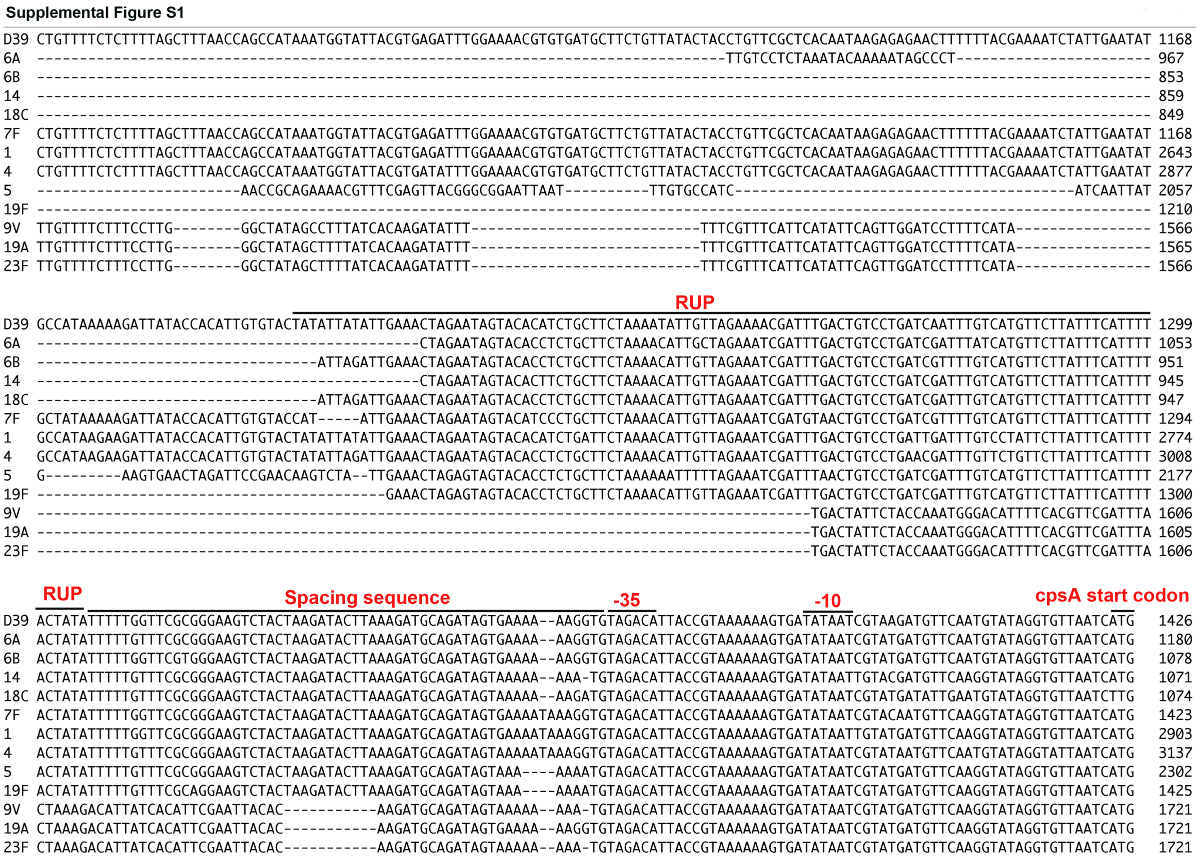
Supplemental Figures and Table

Figure S1. Alignment of the sequences upstream of *cpsA* between D39 and 12 Wzy-dependent CPS types of Streptococcus pneumoniae covered by the 13-valent childhood vaccine.



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D39 CTGTTTTCTCTTTTAGCTTTAACCAGCCATAAATGGTATTACGTGAGATTTGGAAAACGTGTGATGCTTCTGTTATACTACCTGTTCGCTCACAATAAGAGAGAACTTTTTTACGAAAATCTATTGAATAT 1168
6A  ---------------------------------------------------------------------------------TTGTCCTCTAAATACAAAAATAGCCCT----------------------- 967
6B  ----------------------------------------------------------------------------------------------------------------------------------- 853
14  ----------------------------------------------------------------------------------------------------------------------------------- 859
18C ----------------------------------------------------------------------------------------------------------------------------------- 849
7F  CTGTTTTCTCTTTTAGCTTTAACCAGCCATAAATGGTATTACGTGAGATTTGGAAAACGTGTGATGCTTCTGTTATACTACCTGTTCGCTCACAATAAGAGAGAACTTTTTTACGAAAATCTATTGAATAT 1168
1   CTGTTTTCTCTTTTAGCTTTAACCAGCCATAAATGGTATTACGTGAGATTTGGAAAACGTGTGATGCTTCTGTTATACTACCTGTTCGCTCACAATAAGAGAGAACTTTTTTACGAAAATCTATTGAATAT 2643
4   CTGTTTTCTCTTTTAGCTTTAACCAGCCATAAATGGTATTACGTGATATTTGGAAAACGTGTGATGCTTCTGTTATACTACCTGTTCGCTCACAATAAGAGAGAACTTTTTTACGAAAATCTATTGAATAT 2877
5   ------------------------AACCGCAGAAAACGTTTCGAGTTACGGCGGAATTAAT-----------TTGTGCCATC----------------------------------------ATCAATTAT 2057
19F ----------------------------------------------------------------------------------------------------------------------------------- 1210
9V  TTGTTTTCTTTCCTTG--------GGCTATAGCCTTTATCACAAGATATTT---------------------------TTTCGTTTCATTCATATTCAGTTGGATCCTTTTCATA---------------- 1566
19A TTGTTTTCTTTCCTTG--------GGCTATAGCTTTTATCACAAGATATTT---------------------------TTTCGTTTCATTCATATTCAGTTGGATCCTTTTCATA---------------- 1565
23F TTGTTTTCTTTCCTTG--------GGCTATAGCTTTTATCACAAGATATTT---------------------------TTTCGTTTCATTCATATTCAGTTGGATCCTTTTCATA---------------- 1566

D39 GCCATAAAAAGATTATACCACATTGTGTACTATATTATATTGAAACTAGAATAGTACACATCTGCTTCTAAAATATTGTTAGAAAACGATTTGACTGTCCTGATCAATTTGTCATGTTCTTATTTCATTTT 1299
6A  ---------------------------------------------CTAGAATAGTACACCTCTGCTTCTAAAACATTGCTAGAAATCGATTTGACTGTCCTGATCGATTTATCATGTTCTTATTTCATTTT 1053
6B  ---------------------------------ATTAGATTGAAACTAGAATAGTACACCTCTGCTTCTAAAACATTGTTAGAAATCGATTTGACTGTCCTGATCGTTTTGTCATGTTCTTATTTCATTTT 951
14  ---------------------------------------------CTAGAATAGTACACTTCTGCTTCTAAAACATTGTTAGAAATCGATTTGACTGTCCTGATCGATTTGTCATGTTCTTATTTCATTTT 945
18C ---------------------------------ATTAGATTGAAACTAGAATAGTACACCTCTGCTTCTAAAACATTGTTAGAAATCGATTTGACTGTCCTGATCGATTTGTCATGTTCTTATTTCATTTT 947
7F  GCTATAAAAAGATTATACCACATTGTGTACCAT-----ATTGAAACTAGAATAGTACATCCCTGCTTCTAAAACATTGTTAGAAATCGATGTAACTGTCCTGATCGTTTTGTCATGTTCTTATTTCATTTT 1294
1   GCCATAAGAAGATTATACCACATTGTGTACTATATTATATTGAAACTAGAATAGTACACATCTGATTCTAAAACATTGTTAGAAATCGATTTGACTGTCCTGATTGATTTGTCCTATTCTTATTTCATTTT 2774
4   GCCATAAGAAGATTATACCACATTGTGTACTATATTAGATTGAAACTAGAATAGTACACCTCTGCTTCTAAAACATTGTTAGAAATCGATTTGACTGTCCTGAACGATTTGTTCTGTTCTTATTTCATTTT 3008
5   G---------AAGTGAACTAGATTCCGAACAAGTCTA--TTGAAACTAGAGTAGTACACCTCTGCTTCTAAAAAATTTTTAGAAATCGATTTAACTGTCCTGATCGATTTGTCATGTTCTTATTTCATTTT 2177
19F -----------------------------------------GAAACTAGAGTAGTACACCTCTGCTTCTAAAACATTGTTAGAAATCGATTTGACTGTCCTGATCGATTTGTCATGTTCTTATTTCATTTT 1300
9V  -------------------------------------------------------------------------------------------TGACTATTCTACCAAATGGGACATTTTCACGTTCGATTTA 1606
19A -------------------------------------------------------------------------------------------TGACTATTCTACCAAATGGGACATTTTCACGTTCGATTTA 1605
23F -------------------------------------------------------------------------------------------TGACTATTCTACCAAATGGGACATTTTCACGTTCGATTTA 1606

D39 ACTATATTTTTGGTTCGCGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTGAAAA--AAGGTGTAGACATTACCGTAAAAAAGTGATATAATCGTAAGATGTTCAATGTATAGGTGTTAATCATG   1426
6A  ACTATATTTTTGTTTCGCGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTGAAAA--AAGGTGTAGACATTACCGTAAAAAAGTGATATAATCGTATGATGTTCAATGTATAGGTGTTAATCATG   1180
6B  ACTATATTTTTGGTTCGTGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTGAAAA--AAGGTGTAGACATTACCGTAAAAAAGTGATATAATCGTATGATGTTCAATGTATAGGTGTTAATCATG   1078
14  ACTATATTTTTGTTTCGCGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTAAAAA--AAA-TGTAGACATTACCGTAAAAAAGTGATATAATTGTACGATGTTCAAGGTATAGGTGTTAATCATG   1071
18C ACTATATTTTTGTTTCGCGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTAAAAA--AAGATGTAGACATTACCGTAAAAAAGTGATATAATCGTATGATATTGAATGTATAGGTGTTAATCTTG   1074
7F  ACTATATTTTTGGTTCGCGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTGAAAATAAAGGTGTAGACATTACCGTAAAAAAGTGATATAATCGTACAATGTTCAAGGTATAGGTGTTAATCATG   1423
1   ACTATATTTTTGGTTCGCGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTGAAAATAAAGGTGTAGACATTACCGTAAAAAAGTGATATAATTGTATGATGTTCAAGGTATAGGTGTTAATCATG   2903
4   ACTATATTTTTGTTTCGCGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTAAAAATAAAGGTGTAGACATTACCGTAAAAAAGTGATATAATCGTATAATGTTCAATGTATAGGTATTAATCATG   3137
5   ACTATATTTTTGTTTCGCGGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTAAA----AAAATGTAGACATTACCGTAAAAAAGTGATATAATCGTATGATGTTCAAGGTATAGGTGTTAATCATG   2302
19F ACTATATTTTTGTTTCGCAGGAAGTCTACTAAGATACTTAAAGATGCAGATAGTAAA----AAAATGTAGACATTACCGTAAAAAAGTGATATAATCGTATGATGTTCAAGGTATAGGTGTTAATCATG   1425
9V  CTAAAGACATTATCACATTCGAATTACAC-----------AAGATGCAGATAGTAAAAA--AAA-TGTAGACATTACCGTAAAAAAGTGATATAATCGTATGATGTTCAAGGTATAGGTGTTAATCATG   1721
19A CTAAAGACATTATCACATTCGAATTACAC-----------AAGATGCAGATAGTGAAAA--AAGGTGTAGACATTACCGTAAAAAAGTGATATAATCGTATGATGTTCAAGGTATAGGTGTTAATCATG   1721
23F CTAAAGACATTATCACATTCGAATTACAC-----------AAGATGCAGATAGTAAAAA--AAA-TGTAGACATTACCGTAAAAAAGTGATATAATCGTATGATGTTCAAGGTATAGGTGTTAATCATG   1721
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Figure S2. Impact of the TIGR4 SS element on the *cps* transcription.

A. Quantification of the *cps2A* mRNA in the *dexB-cps4A* swap strains of D39s by qRT-PCR

B. Image of a representative CPS immunoblot.

C. Quantitative chemiluminescence intensity levels from the immunoblotting results represented in (B).

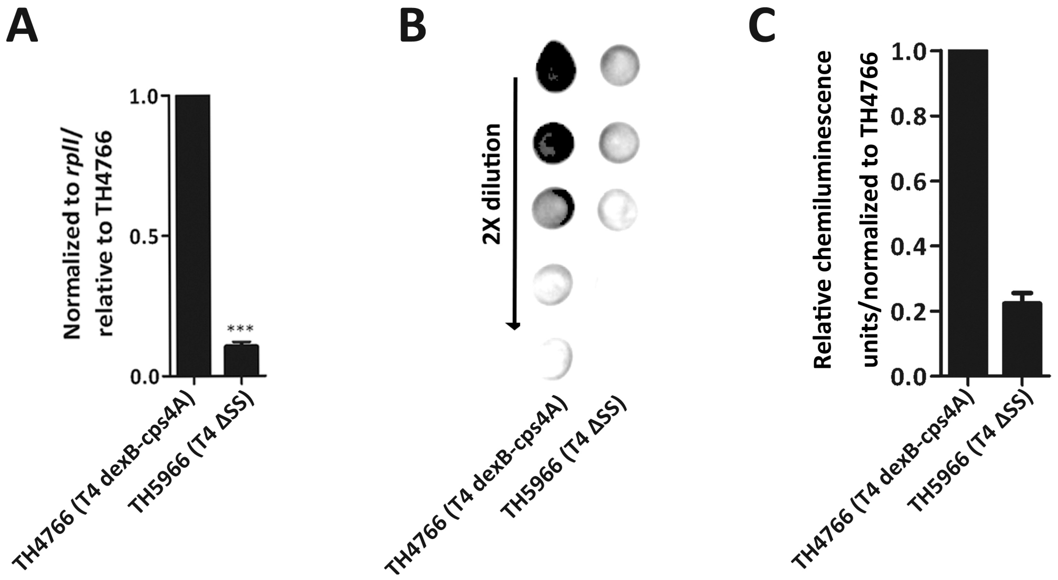

Table S1. The primers used in this study

Primer ID	Sequence (5’-3’)
Pr74	CATCGATACAGAGAACGAGGGAAGTAC
Pr387	TGTTCCCAGCTATTTTTATTCAGA
Pr388	TCTCTTTATCCCCTTTCCTTATGC
Pr646	TAGTGCAGGCCTGACTTTTG
Pr649	CTGAGAAGGTGTGAATGGACGA
Pr652	GACTTCCCGCGAACCAAAATAATATAGTACACAATGTG
Pr653	CACATTGTGTACTATATTATTTTGGTTCGCGGGAAGTC
Pr758	ACGATCTGTCTTGATGTTTTCTAA
Pr894	CACGTTCACAGAAAGTGAAGC
Pr895	CTGCGATTGGTACATAGGC
Pr972	GAGAGGATCCTATGTCTTTGACGGTAAGAAACC
Pr973	GAGACTCGAGTACTACTTATCTCACTTCTTGTTTGTAAAATTC
Pr1025	5'-GAGAGGATCCACTTCGCTTCACTTTCTGTGAAC-3'
Pr1026	5'-GAGAAAGCTTGCTTCTGTTATACTACCTGTTCGC-3'
Pr1168	GATAGACGTCCATTCGCATATCG
Pr1169	GTTCCTGAGCCTTCGCTTCTC
Pr1170	CTTGTGGAAACGCAAGCTAATG
Pr1171	GTCCAAATCTGGAACGAGCC
Pr1172	GGAAGTTATGCCAACGTTAGAAATC
Pr1173	CCCGTAATCTCCATAAGAACCG
Pr1276	GACCAATTTTCGTGATACGAGGAT
Pr5791	ATAGAGCTCCTACGGGGATCTTACAATTTGGG
Pr6212	GAGAGGATCCCAAATCTGTCTTGATTGAAAACA
Pr6213	GAGACCCGGGCATTGAACATCTTACGATTATATCACT
Pr6214	GAGACCCGGGTAATTAGCTGAAGGAGGAATAATG
Pr6754	TGTATAGCTGCTTTAACTACCTTCACTTTCTGTGAACGTG
Pr6755	CACGTTCACAGAAAGTGAAGGTAGTTAAAGCAGCTATACA
Pr6756	ATTTTTCGAGGATTGTCTATGTCATCTACATCAAAAACGA
Pr6757	TCGTTTTTGATGTAGATGACATAGACAATCCTCGAAAAAT
Pr6758	GGAACGAGCCCTAAAAGTGGTAATTGAAATACATCGATTT
Pr6759	AAATCGATGTATTTCAATTACCACTTTTAGGGCTCGTTCC
Pr6760	TCTCCATAAGAACCGTATTTTACAAAATCCAATTTTGCCT

Pr6761	AGGCAAAATTGGATTTTGTAAAATACGGTTCTTATGGAGA
Pr6850	CACGTTCACAGAAAGTGAAGCTCGAGCCGTTTGATTTTTA
Pr6851	TAAGAACCGTATTTTCTAGAGGCCCCTTTCCTTATGCTTT
Pr6852	TAAAAATCAAACGGCTCGAGCTTCACTTTCTGTGAACGTG
Pr6853	AAAGCATAAGGAAAGGGGCCTCTAGAAAATACGGTTCTTA
Pr6854	TCTGCCAAAGCCTTTGCAATCTTGC
Pr7022	TCTCCATAAGAACCGTATTTCTTCACTTTCTGTGAACGTG
Pr7023	CACGTTCACAGAAAGTGAAGAAATACGGTTCTTATGGAGA
Pr7177	TCTAGTTTCAATATAATATACAAAAAGCACCTCAAAAAGGTATTACC
Pr7178	CCTTTTTGAGGTGCTTTTTGTATATTATATTGAAACTAGA
Pr7179	GACTTCCCGCGAACCAAAAACAAAAAGCACCTCAAAAAGGTATTACC
Pr7180	CCTTTTTGAGGTGCTTTTTGTTTTTGGTTCGCGGGAAGTC
Pr7181	CTTTTTTACGGTAATGTCTACAAAAAGCACCTCAAAAAGGTATTACC
Pr7182	CCTTTTTGAGGTGCTTTTTGTAGACATTACCGTAAAAAAG
Pr7183	ACTCATGATTAACACCTATACAAAAAGCACCTCAAAAAGGTATTACC
Pr7184	CCTTTTTGAGGTGCTTTTTGTATAGGTGTTAATCATGAGT
Pr7339	TTCCTGACGAGAAGGTAGTCAATAA
Pr7344	GTCTAGATGGACATTCCCTACTGGG
Pr7345	AAAGCATAAGGAAAGGGGCCTTATAGTAATTCCACACAGA
Pr7346	TCTGTGTGGAATTACTATAAGGCCCCTTTCCTTATGCTTT
Pr7347	ACTCATGATTAACACCTATACCGTTTGATTTTTAATGGAT
Pr7348	ATCCATTAAAAATCAAACGGTATAGGTGTTAATCATGAGT
Pr7705	CGCTCAGTGTCGCTGTTTTA
Pr7706	TCTCCCCTGCAATCAAACTC
Pr7707	TGATATCATGGGTGCATTGG
Pr7708	TCCACCCTGCATGGTATTTT
Pr7709	CGACCATCTGGACCAACTTT
Pr7710	CTAGCCAAAGAAGCGACTGC
Pr8067	GGTCAATGCTCAGTTAGAAAGTG
Pr8068	GATATGCGAATGGACGTCTATC
Pr8069	TGGTTCATGTCATTGCAAGTG
Pr8070	TGAAATACATCGATTTCTATCGTG
Pr8071	GACGCAATTCCTTGTTTGG
Pr8072	GCCTGTGAGATTTCTAACGTTG
Pr8073	TTTAGTGACGGAGGCAGGTG

Pr8074	TGGATTATGGCCAATGAAGAC
Pr8075	AGGTCAGCGGACGAAGTG
Pr8076	GCAGGAATCCCCTTTGAAC
Pr8077	CGGAGAGAATGGTGCCATC
Pr8078	AGCTCCATTATAAGTTGCCATC
Pr8079	TTCGTGAATTTATCAGCATTCCAC
Pr8080	GATAACTCTTATTCCACCTGTCATTCTC
Pr8081	GAGGATCGATTATTGTTGGCAC
Pr8082	ACAGGAGTAACACCATTTCCTG
Pr8083	GTGGTTATTATCTGTGCATTTATAGG
Pr8084	GGGTAGCTCCCGATGATATATC
Pr8085	GAACCAGGAGATTATATGCAGC
Pr8086	CCAAAGTTGCTTAAAGTTAGTAATCC
Pr8087	ATAGTTATATTCTTTATCCTATACAAGTTGTG
Pr8088	CTGCAATGGATAGACCAACATATC
Pr8089	CGTCAAAGCTCTATTCAAGGTATTATG
Pr8090	ATTGCTTCTCAGTATGGACCGC
Pr8091	AATACCACGAAGAAGCTTCACAG
Pr8092	GTAAGTGGATACAGACGGGTACC
Pr8093	GCCTCTCAATATATTGAAACGG
Pr8094	GGCATCAATTTTACGCACC
Pr8095	CAAGTATGCCTTTGTGAACTACGC
Pr8096	GATAAAGCCAGCTCCACCTGTC
Pr8097	CTTTGAAGCTGGGCTCAAG
Pr8098	TCCTAATTGGCCATTTGCC
Pr8105	CCCAAATGGAAGCATAAAGAG
Pr8106	ACTTCCCATAACAAGACTGGC
Pr8584	GACTTCCCGCGAACCAAAAAGTACACAATGTGGTATAATC
Pr8585	GATTATACCACATTGTGTACTTTTTGGTTCGCGGGAAGTC
Pr8586	CTTTTTTACGGTAATGTCTATATAGTAAAATGAAATAAGA
Pr8587	TCTTATTTCATTTTACTATATAGACATTACCGTAAAAAAG
Pr8588	ACTCATGATTAACACCTATACACCTTTTTTCACTATCTGC
Pr8589	GCAGATAGTGAAAAAAGGTGTATAGGTGTTAATCATGAGT
Pr8590	CTTTTTTACGGTAATGTCTAGTACACAATGTGGTATAATC
Pr8591	GATTATACCACATTGTGTACTAGACATTACCGTAAAAAAG

Pr9638	TGCAGTGGTACCTAGGAATCTAGACATTACCGTAAAAAAGTGATAT
Pr9639	GATTCCTAGGTACCACTGCAATCTTTAAGTATCTTAGTAGACTTCCCGC
Pr9641	TGCAGTGGTACCTAGGAATCGCAGATAGTGAAAAAAGGTGTAGACATT
Pr9642	GATTCCTAGGTACCACTGCAACTTCCCGCGAACCAAAAATATAGTAA
Pr9643	GCAGTGGTACCTAGGAATCCTACTAAGATACTTAAAGATGCAGATAGT
Pr9644	GATTCCTAGGTACCACTGCTATAGTAAAATGAAATAAGAACATGACAAAT
Pr9645	GATAGTGCCTAGGAATCTAGACATTACCGTAAAAAAGTGATATAATCGTAAG
Pr9646	CGGTAATGTCTAGATTCCTAGGCACTATCTGCATCTTTAAGTATCTTAGT
Pr9647	ACTTAAAGATTGCAGTGGTAAAAAAAGGTGTAGACATTACCGTAAAAAAGTGA
Pr9648	CCTTTTTTTACCACTGCAATCTTTAAGTATCTTAGTAGACTTCCCGCGAACC
Pr9649	TTATTTCATTTTACTATATAGACATTACCGTAAAAAAGTGATAT
Pr9650	TTTTACGGTAATGTCTATATAGTAAAATGAAATAAGAACATGAC