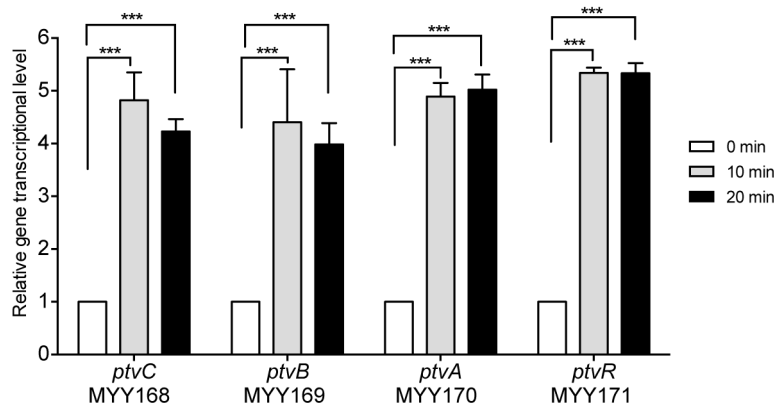
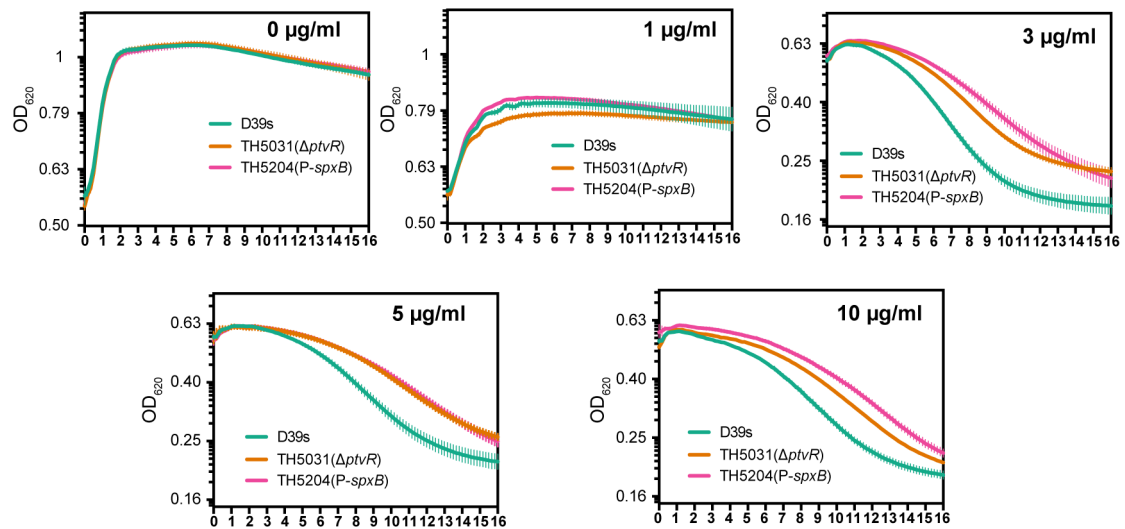


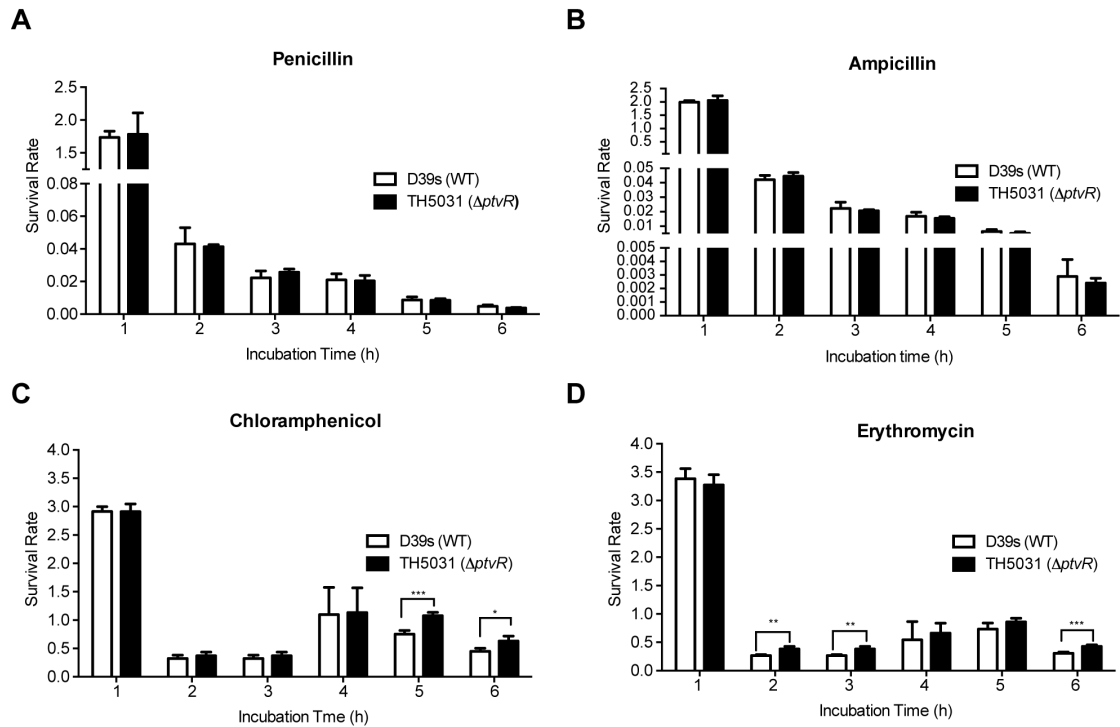
## SUPPLEMENTARY FIGURES



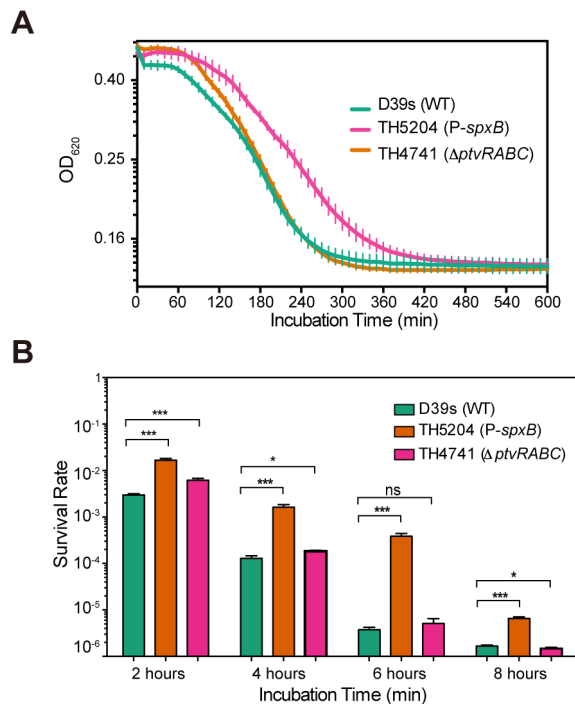
**Figure S1.** The mRNA levels of the *ptv* genes in *S. pneumoniae* ST556 before and after vancomycin treatment as described in Figure 1B. The gene locus MYY171-168 represents *ptvR-C*, respectively.



**Figure S2.** Impact of drug concentration on the *ptv*-mediated vancomycin tolerance of *S. pneumoniae*. Pneumococcal strains were processed as described in Fig. 4A, except that different concentrations of vancomycin were used as indicated in each sub-figure.



**Figure S3. Impact of the *ptv* operon on pneumococcal tolerance to other antibiotics.** Tolerance of D39s and isogenic *ptvR*-null mutant (TH5031) to penicillin (A) (0.12  $\mu\text{g/ml}$ ), ampicillin (B) (0.5  $\mu\text{g/ml}$ ), chloramphenicol (C) (10  $\mu\text{g/ml}$ ), and erythromycin (D) (0.6  $\mu\text{g/ml}$ ) were determined by mixing each compound with mid-log phase cultures to a final concentration of its 10-fold MIC value. Viable pneumococci were enumerated by colony plating and presented as in Fig. 4B or 4C.



**Figure S4. Impact of pretreatment with a lower dose of vancomycin on the tolerance of wild type *S. pneumoniae* D39 to a lethal concentration of vancomycin.**

**A. Impact of prior treatment with vancomycin on vancomycin tolerance of D39 as assessed by pneumococcal autolysis (culture turbidity).** D39s and its derivatives with insertion of the *spxB* promoter upstream of *ptvR* (TH5204), and deletion of the *ptv* operon (TH4741) were cultured in THY broth to OD<sub>620</sub> 0.4. The cultures were pretreated with 0.5 µg/ml vancomycin for 20 min to induce the expression of the *ptv* operon, and subsequently mixed with vancomycin to a final concentration of 5 µg/ml. The culture turbidity was monitored by spectrophotometry immediately before (0 h) and after addition of vancomycin every 10 minutes for 10 hours.

**B. Impact of prior treatment with vancomycin on vancomycin tolerance of D39 as assessed by bacterial viability.** Pneumococcal strains were processed as in (A) except for plating the cultures on blood agar dishes at various time points before (0 h) and after (1-8 h) the addition of vancomycin. Survival rate represents the CFU ratio of the same cultures before and after addition of vancomycin. Asterisks indicate significant increase of strains TH5204 and TH4741 over D39s in survival rate after vancomycin treatment (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ).

**Supplemental Table S1. The Primers used in this work**

<b>Primers</b>	<b>Sequence (5'-3')</b>
Pr1097	GAGATCTAGACCGTTTGATTTTAAATGGATAATG
Pr1098	GAGACTCGAGCCTTTCCTTATGCTTTTGGAC
Pr2773	ATAGGATCCGGATTCTCATCTTTGGTGTG
Pr2774	AGTGAATTCAACGTCTGCTTGGTGTGGA
Pr3129	ATAGCGGCCGCACTCAAGGGAAAATTACTGCAT
Pr3130	AGTGGCGCGCCGAATAAAGTCTACAAGTTTCATATTGA
Pr3110	GATCCTCTAGAAGCGGCCCATGGTACCGGGCGCGCCCTCGAGGCTAGCG
Pr3111	AATTCGCTAGCCTCGAGGGCGCGCCCGGTACCATGGCGGCCGCTTCTAGAG
Pr7187	CACCTACAGCCTCTTCTTCTTGA
Pr7188	GAGATCTAGAGGGAAACCTCCTTTTCTAATGT
Pr7189	GAGACTCGAGTCATAGAAGGGAGTATCCGCC
Pr7190	TGGTAGTCGGAGAATAACTTCTTC
Pr7195	TCGCTTCTTCGGTCTACTCA
Pr7196	GAGATCTAGAGGTAGAACCTCCTTTTATGTTAGATAAA
Pr7197	GAGACTCGAGAATTCAATCCTGTCATCCTTGG
Pr7198	GCTTGGATCAGGCAGCTACT
Pr7207	TCAGTTCTTGTTCATGGCGGGATGTTGGAAAGTACAT
Pr7208	ATGTACTTCCAACATCCCGCCATGACAAGAAGTGA
Pr7212	TTAAAGAAAGGCCAAGATAAACCATCTTGTTCAT
Pr7213	ATGAAACAAGAATGGTTTATCTTGGCCTTTCTTTAA
Pr7275	CAAGGACAGACAAGAATAATACGGCCAAGATGAGAAAT
Pr7276	ATTTCTCATCTTGGCCGTATTATTCTTGTCTGTCCTTG
Pr7878	GAGTGTCCATTCATCTTTTAGGGTC
Pr7879	TCCTCTGCCTTGATTGAATTTCTC
Pr7880	GGAGGGCTAACAAGGCGATATG
Pr7881	CGCCATCCTCGTGACCCT
Pr7882	GGTGAGCAGAAAGAGATGGATGG
Pr7883	CCAAGTCCATGGTAAGGTTGAACT
Pr7884	CTCGCTTCACTGTCTTCAGGAGTT
Pr7891	GATACGACTTCCTTCAATTCGGAG
Pr7893	CACAACGAAACTACCCAAGAGGAA
Pr7932	GATTGCCATCATGAGTGACAAGG
Pr7933	AGTGTCCACTTCGCGAAGGGT
Pr8059	CTGGTGTGATCGGCATTTCC
Pr8060	GAGATCTAGAGGGAAACCTCCTTTTCTAATGTGTAAG
Pr8061	GAGACTCGAGATGTACTTTCCAACATCCTCTGCC
Pr8062	GGGCCGCTTCTTTGGGAGTT
Pr8064	TGAAGGAGAGTTATCATTATGTACTTTCCAACATCC
Pr8065	GGATGTTGGAAAGTACATAATGATAACTCTCCTTCA

Pr8121	GAGACTCGAGGGGAAACCTCCTTTTCTAATGTGTAAG
Pr8139	GAGACTCGAGAAGTAATGACTAGATTTCTTTGTTATAA
Pr8171	GAGACATATGTACTTTCCAACATCCTCT
Pr8172	GAGACTCGAGGTTCTTGTCATGGC
Pr8201	CTTGCTCATTCTGTTTTCTGC
Pr8202	GCACCGATAGCCAGTAAACC
Pr8203	GGGATTCTTCATATCGCAAGTA
Pr8513	GAGATCTAGACATGCGTAAATGGACAAAAGGA
Pr8514	AGTTCAACCTTACCATGGACTTGG
Pr8515	ACTCTATACAGACACCATCAATGGCA
Pr8516	GAGATCTAGATCCTTTTGTCCATTTACGCATG
Pr8517	GAGACTCGAGATATCCACACCAAGCAGACGTTGA
Pr8518	TAGCTGCTCAGGGAAGCGAC
Pr8521	CGTAAATGGACAAAAGGAATATCCACACCAAGCAGA
Pr8522	TCTGCTTGGTGTGGATATTCCTTTTGTCCATTTACG
Pr8547	AGAACTGAATACCTGACTCTCATTGTTCTGTCTGGTA
Pr8548	TACCAGACGAACAATGAGAGTCAGGTATTCAGTTCT
Pr8550	GAGACTCGAGTCAGTTCTTGTCATGGCGGG
Pr8551	ACACCGTCATGACCTGGTTACC
Pr10024	CCCTGCTCCAGTACGGCC
Pr10025	CCTTACAGTGGACAGAGCGGATAAG
Pr10379	GAGAGGGCCCAAGTAATGACTAGATTTCTTTGTTATAAAAC
Pr10380	GACCCGGGTCAGTGGTGGTGGTGGTGGTGGTCTTGTCATGGCGGATAAC
Pr10655	CCTTACAGTGGACAGAGCGGATAAGATCCTTTAAACTTCTAGTTTT
Pr10656	AAAAGTAGAAGTTTAAAGGATCTTATCCGCTCTGTCCACTGTAAAG
Pr10657	AGCTAGAAGTTTTTATATATAAAAATTTTACACAT
Pr10658	ATGTGTAAAATTTTTATATATAAAAAACTTCTAGCT
Pr10659	TTCTCTTGACAGGGCTTTCTTTTAGATGTACAATGT
Pr10660	ACATTGTACATCTAAAAGAAAGCCCTGTCAAGAGAA
Pr10661	ATGTGTAGAAAATTTATATATAAAAATCTTACACAT
Pr10662	ATGTGTAAGATTTTTATATATAATTTTTCTACACAT
Pr10663	TAGAAAAGGAGGTTTCCCATGTACTTTCCAACATCCTCTGCCTTGATTGAATT TCTCATCTTGGCCGTAAGGAGCAGGG
Pr10664	CCCTGCTCCAGTACGGCCAAGATGAGAAATTCAATCAAGGCAGAGGATGTT GGAAAGTACATGGGAAACCTCCTTTTCTA
Pr10379	GAGAGGGCCCAAGTAATGACTAGATTTCTTTGTTATAAAAC
Pr10380	GACCCGGGTCAGTGGTGGTGGTGGTGGTGGTCTTGTCATGGCGGATAAC
Pr10383	GATTACGCCAAGCTTTAGGGTCAAGAGCTGCTCTATACCACCG
Pr12560	GCATGCGGCCGCTACACATTAGAAAAGGAGGTTTCCC
Pr12561	GCGCACTAGTGTCTTGTCATGGCGGATACTCC
Pr12562	GCATGCGGCCGCGGCATCATAGAAGGGAGTATCCG
Pr12563	GCGCACTAGTCGCATGCTGATTACCTCTCTTTC

Pr12564	GCATGCGGCCGCAAAAAAGGAAAGAGAGGTAATCAGCAT
Pr12565	GCGCACTAGTACGTCTGCTTGGTGTGGATATTAG
Pr12566	GCATGCGGCCGCATCTAACTAAAAAGGAGGTTCTACCATG
Pr12567	GCGCACTAGTAAGAAAAGGCCAAGATACGAAGATAA
Pr12568	GCGCACTAGTTACTTTCCAACATCCTCTGCCTTG
Pr12569	GCATGCGGCCGCTCAGTTCTTGTCATGGCGGATA
Pr12570	GCGCACTAGTACAAGAACTGAATACCTGACTCAGCT
Pr12571	GCATGCGGCCGCTTACGCATGCTGATTACCTCTCTTT
Pr12572	GCGCACTAGTCGTAAATGGACAAAAGGATTTCTC
Pr12573	GCATGCGGCCGCTCAACGTCTGCTTGGTGTGGATA
Pr12574	GCGCACTAGTAAACAAGAATGGTTTGAAAGTAATG
Pr12575	GCATGCGGCCGCTTAAAGAAAGGCCAAGATACGAAGA
Pr12576	AATAGCGGCCGCGACAGTTGCGGATGTACTTC
Pr12577	GCGAATTCGATTAATCAGATCTAATTAGCTGAAGGAGGAATAATG
Pr12578	GGAAGTCTCTCAAGCTGAAGTCG
Pr12579	GCATAGATCTTTAAAGAAAGGCCAAGATACGAAG
Pr12580	GCATGCGGCCGCAATTCATCCTGTCACTTGGC
Pr12581	AAACGATTTCAAAGGAGTCCAGTT
Pr12582	GAGAGAATTCACACATTAGAAAAGGAGGTTTCCC
Pr12583	GCGCACTAGTGCTGAGTCAGGTATTCAGTTCTTGTC
Pr12584	AAGTAATGACTAGATTTCTTTGTTATAA
Pr12585	AATGATAACTCTCCTTCAATTTTTTTA
Pr12588	CCCTGCTCCAGTACGGCC (5' end labeled with Cy3)