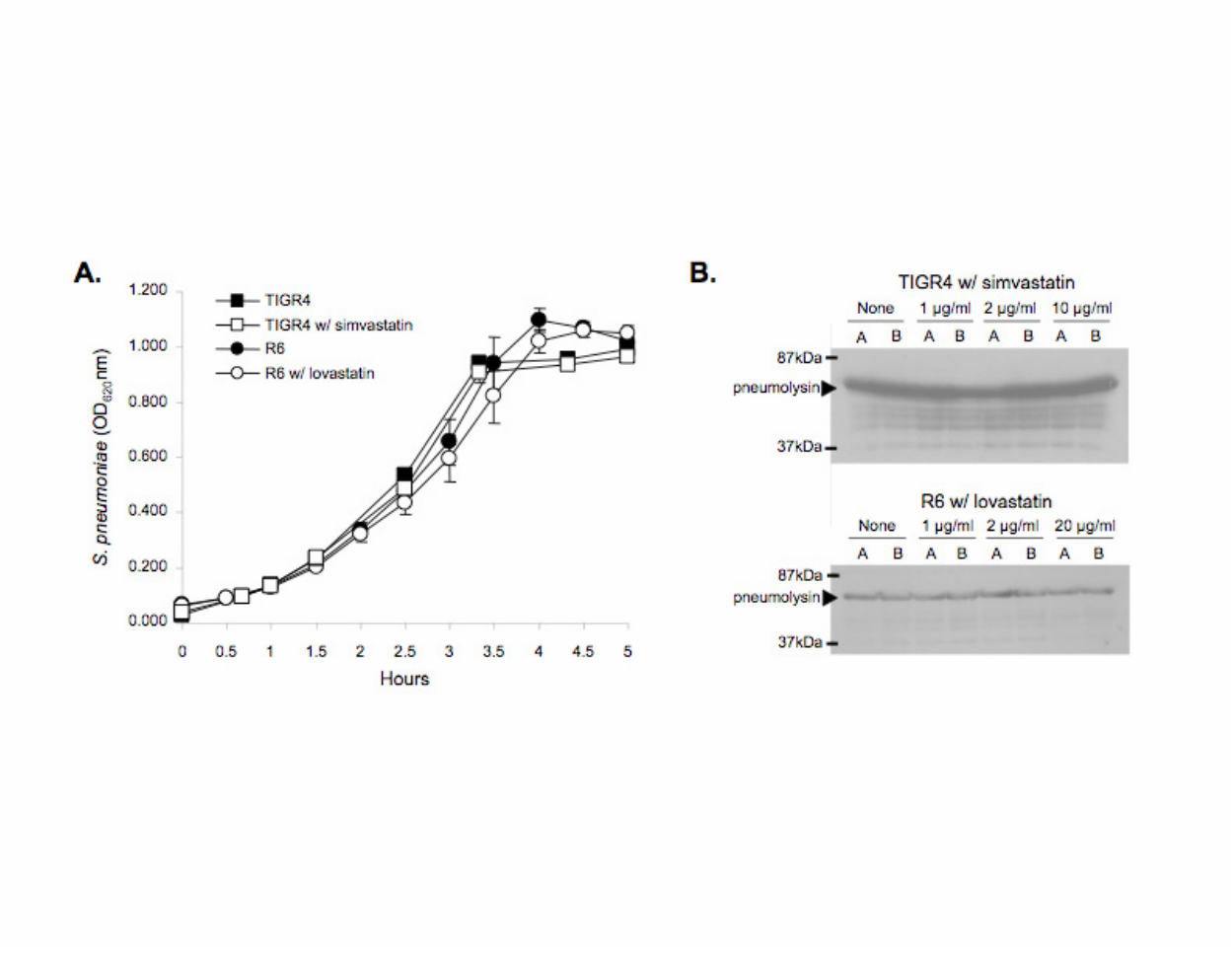
Supplementary Data

Supplementary Table 1. Microarray analysis of statin effect on pneumococcal gene expression. Bacterial RNA was harvested from mid-log phase cultures using Qiagen RNAeasy minikit. Microarray experiments were performed as described previously (1). Briefly, whole-genome *S. pneumoniae* cDNA microarrays were kindly provided from the Pathogen Function Genomics Research Center (PFGRC) at the Institute for Genomic Research (TIGR) consisted of PCR products representing segments of the 2,131 open reading frames of strain TIGR4. Microarray experiments were performed by the Functional Genomics lab, Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children's Research Hospital using standard protocols provided by PFGRC (http://pfgrc.tigr.org/protocols.shtml). Data represents the mean from triplicate experiments using independent RNA samples for genes showing greater than two-fold differences in transcript abundance. If a gene is not listed in the table, there was no significant difference in transcript levels between the control and experimental groups.

Supplementary Table 1

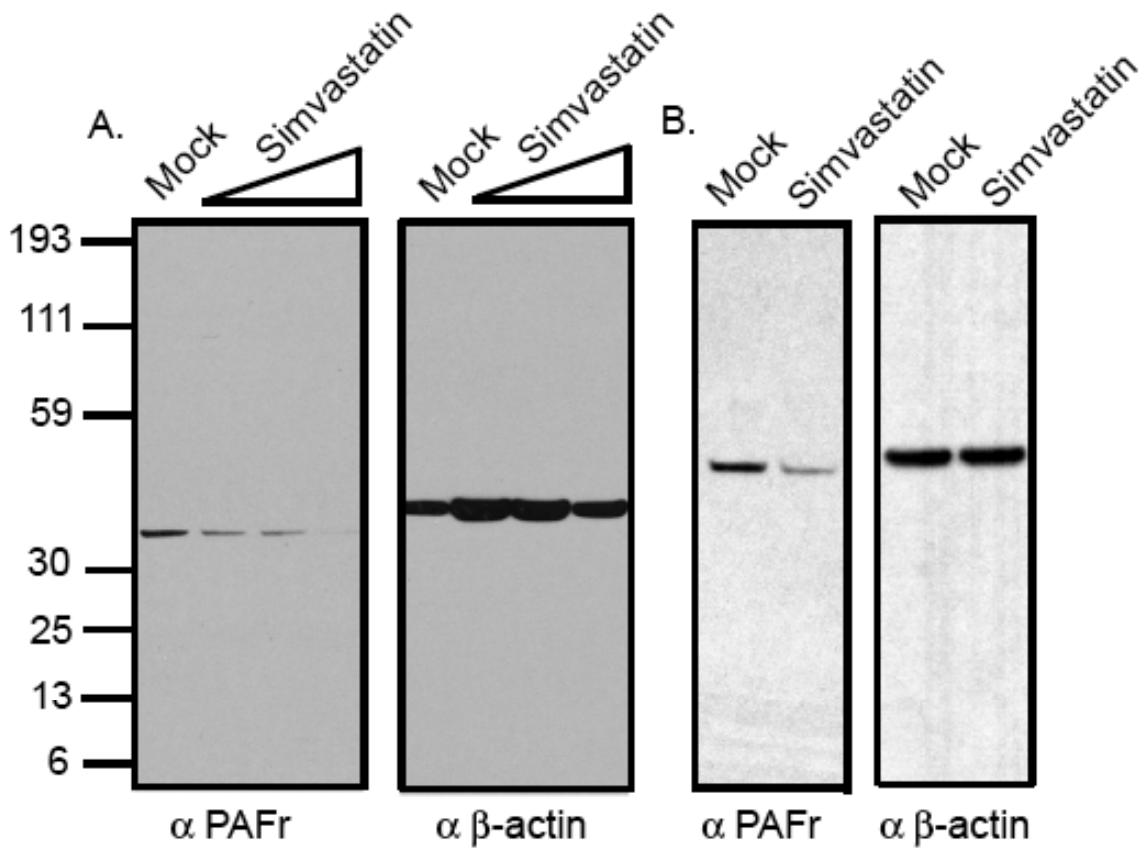
TIGR4 with 10 µg/ml simvastatin:		
Down-regulated genes		
TIGR4 Name	**Common Name of Primary Target**	**Av Fold Change**
SP0501	transcriptional regulator, MerR family	-2.97
SP0502	glutamine synthetase, type I	-2.56
SP0503	hypothetical protein	-2.15
SP1648	manganese ABC transporter, ATP-binding protein	-3.21
SP1649	manganese ABC transporter, permease protein, putative, authentic frameshift	-2.32
SP1650	manganese ABC transporter, manganese-binding adhesion liprotein	-3.37
Up-regulated genes		
TIGR4 Name	**Common Name of Primary Target**	**Av Fold Change**
SP0916	lysine decarboxylase	4.00
SP0918	spermidine synthase	3.15
SP0919	conserved hypothetical protein	4.06
SP0920	carboxynorspermidine decarboxylase	4.11
SP0921	conserved hypothetical protein	3.19
SP0922	carbon-nitrogen hydrolase family protein	3.86
SP1675	ROK family protein	3.51
SP1677	hypothetical protein	2.24
SP1679	hypothetical protein	2.99
SP1680	sugar ABC transporter, permease protein	2.70
SP1681	sugar ABC transporter, permease protein	2.49
SP1682	sugar ABC transporter, sugar-binding protein	2.42
SP1686	oxidoreductase, Gfo/Idh/MocA family	5.15
SP1687	neuraminidase B	2.46
SP1688	ABC transporter, permease protein	2.26
SP1869	iron-compound ABC transporter, permerase protein	4.60
SP1870	iron-compound ABC transporter, permease protein	6.84
SP1871	iron-compound ABC transporter, ATP-binding protein	4.44
SP1872	iron-compound ABC transporter, iron-compound-binding protein	6.88
SP2084	phosphate ABC transporter, phosphate-binding protein	4.18
SP2085	phosphate ABC transporter, permease protein	5.17
SP2086	phosphate ABC transporter, permease protein	10.36
SP2087	phosphate ABC transporter, ATP-binding protein	6.49
SP2088	phosphate transport system regulatory protein PhoU	5.60

Supplementary Figure 1



Supplementary Figure 1. Lack of effect of simvastatin on pneumococcal growth and toxin secretion. A) Growth of strains TIGR4 and R6 in TH media supplemented with 0, 1, 5, and 10 µM simvastatin or lovastatin was measured by OD_{620} over a 6 hour period. B) Pneumococcal strains TIGR4 and R6 were cultured in the presence of increasing concentrations of statins and the supernatant was assayed for pneumolysin production by Western Blot. Lanes A and B represent replicate cultures from independent experiments.

Supplementary Figure 2



Supplementary Figure 2. Dose response and kinetics of PAFr expression in response to simvastatin. A) HBMEC were treated overnight with 1uM, 2.5uM, and 10 uM simvastatin and PAFr expression was assessed by Western blot. ß actin served as the loading control. **B)** PAFr expression assessed by Western blot after 2 hours of 1μM simvastatin showed downregulation of PAFr comparable to Panel A.

References

1. Orihuela, C.J., Radin, J.N., Sublett, J.E., Gao, G., Kaushal, D., and Tuomanen, E.I. 2004. Microarray analysis of pneumococcal gene expression during invasive disease. *Infection and Immunity* 72:5582-5596.