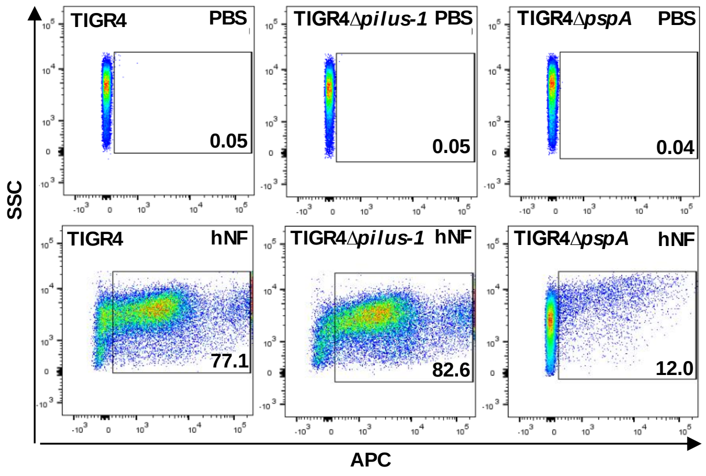
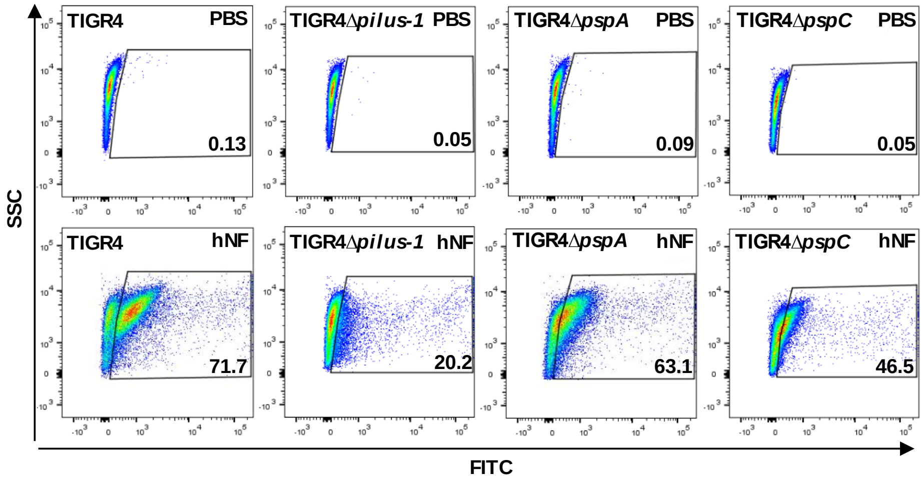
SUPPLEMENTAL FIGURES

Figure S1

Lactoferrin binding



sIgA binding



IgG binding

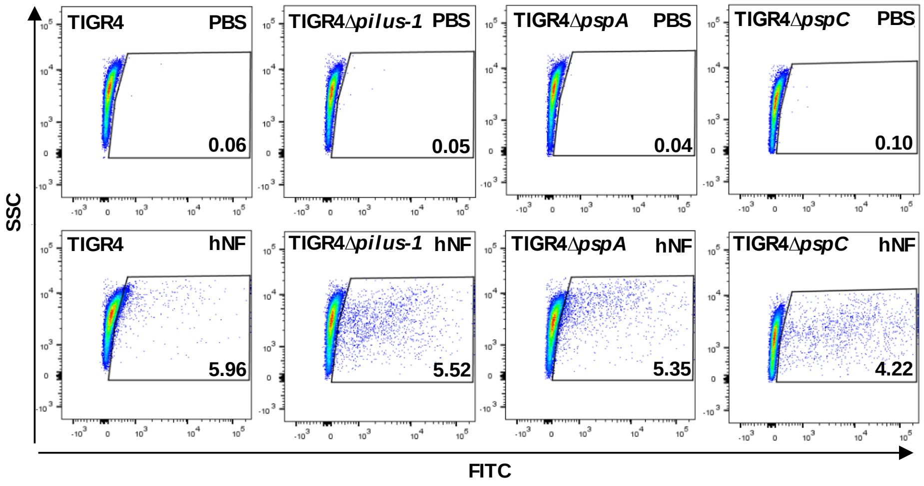


Figure S1: Binding of nasal mucus proteins to *S. pneumoniae*.
Representative dot plots showing the percentage acquisition of soluble LF, sIgA, and IgG from human nasal fluid by Spn. Bacteria (5 x 10^6 CFU/ 50 µl) were incubated with 50 µg/ml of hNF. Binding of bacteria-associated LF, sIgA, and IgG was analyzed by flow cytometry.

Figure S2

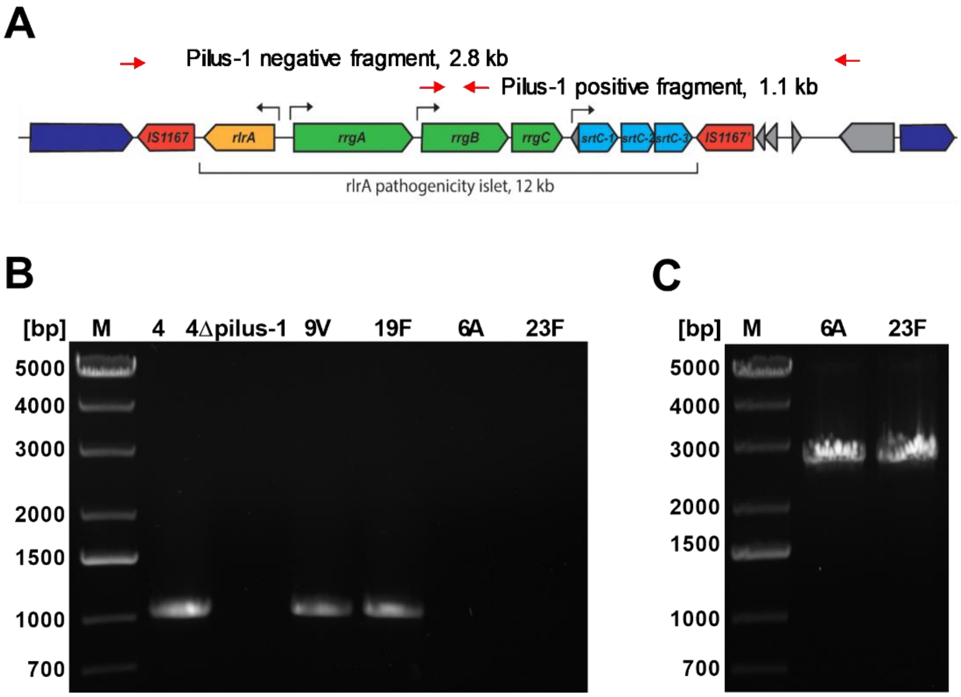


Figure S2: Confirmation of presence and absence of pilus-1 pathogenicity islet in clinical isolates. (A) Schematic model of the gene arrangement within the pilus-1 pathogenicity islet. **(B)** PCR to analyze presence of pilus-1 in Spn using primers within *rrgB* gene generating a 0.9 kb PCR product. **(C)** PCR to confirm absence of pilus-1 in Spn using primers outside the pathogenicity islet generating a 2.8 kb PCR product.

Figure S3

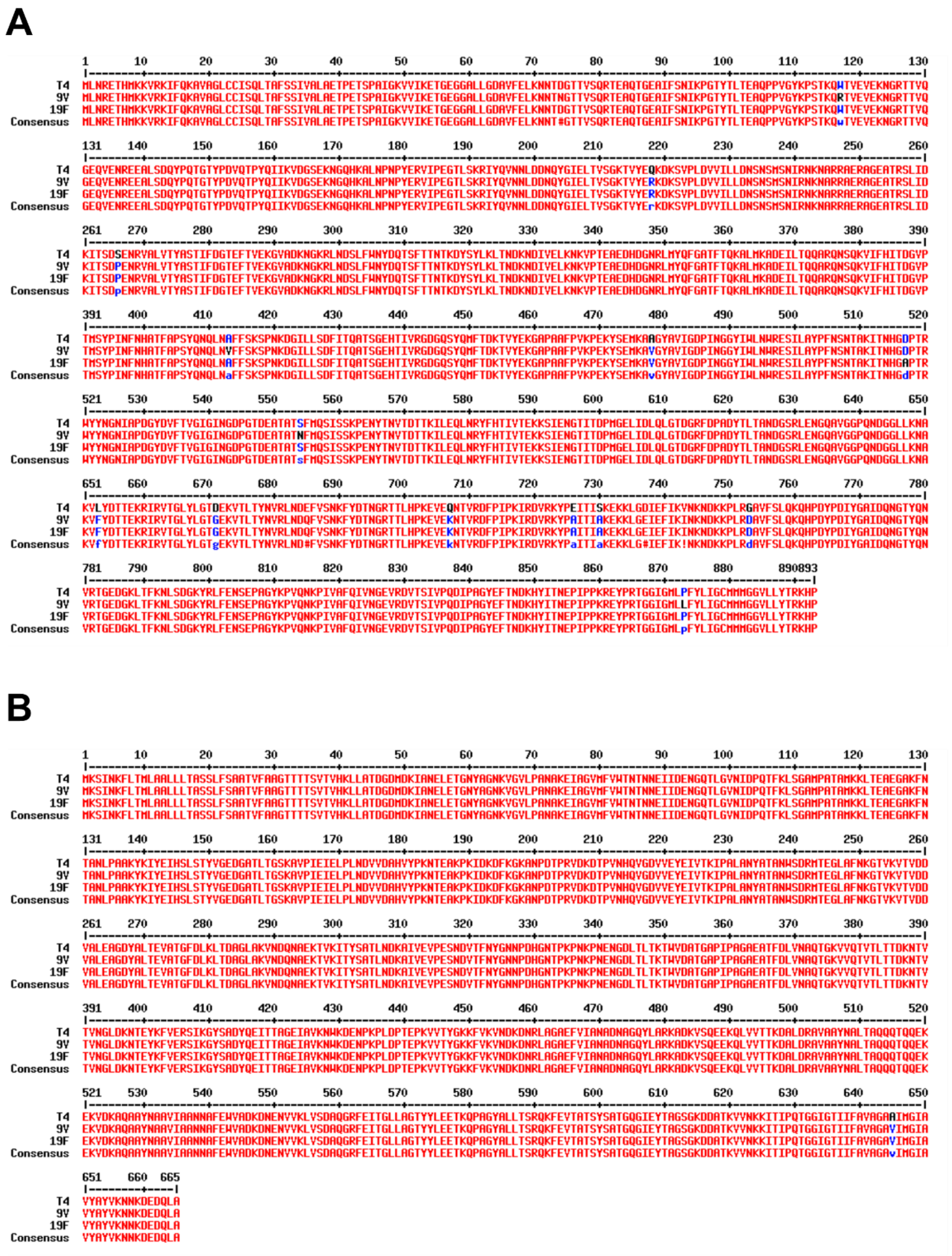


Figure S3: Sequence comparison of RrgA and RrgB of pneumococcal clinical isolates. Alignment of the amino acid sequences of the pilus-1 components RrgA **(A)** and RrgB **(B)** from the pneumococcal strains T4, 9V and 19F using MultAlin (82). Conserved amino acids are illustrated in red. Numbering of the amino acid sequence is shown above the sequences.

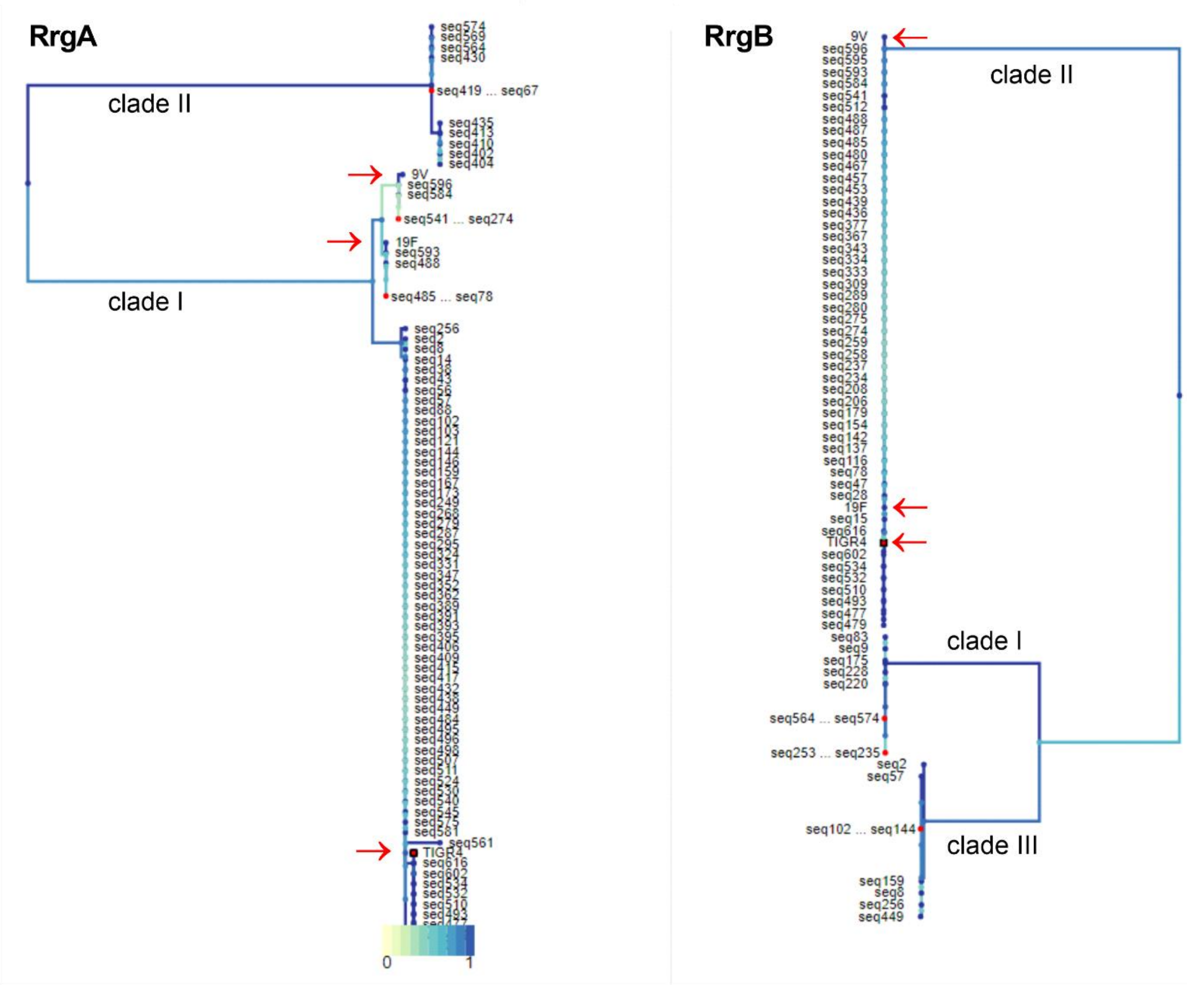


Figure S4: Phylogenetic tree of RrgA and RrgB from pneumococcal carriage isolates in children from Massachusetts (65). Using the alignment of these genes from [Corander et al 2017], we added in sequences from the strains used in the study (highlighted with red arrows). Alignment and tree construction was performed by Seaview v4.0 using MUSCLE and PhyML with default parameters. Trees were midpoint rooted and visualized using phylo.io, with collapsed subclades indicated by red nodes.

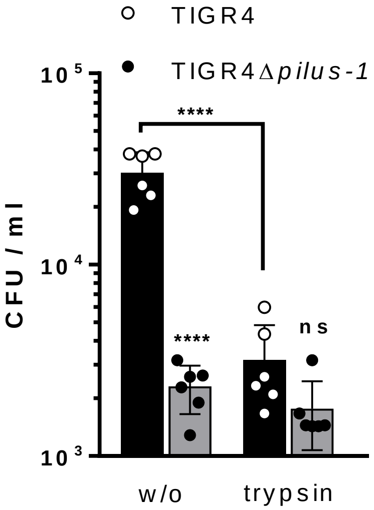


Figure S5: Trypsin reduces adherence to nasal fluid of pilus-expressing S. pneumoniae. Immobilized hNF was incubated with increasing concentrations of 100 µg/ml trypsin for 30 min at 37°C followed by the incubation of 0.1% BSA and 2 x 10^4 Spn TIGR4 in 100 µl DMEM for 2 h at 30°C. Experiments were performed in duplicates and mean values of three independent experiments are shown with error bars corresponding to S.D. **,p<0.01; ****,p<0.0001 by two-tailed unpaired t-test. *n* = 6.

Figure S6

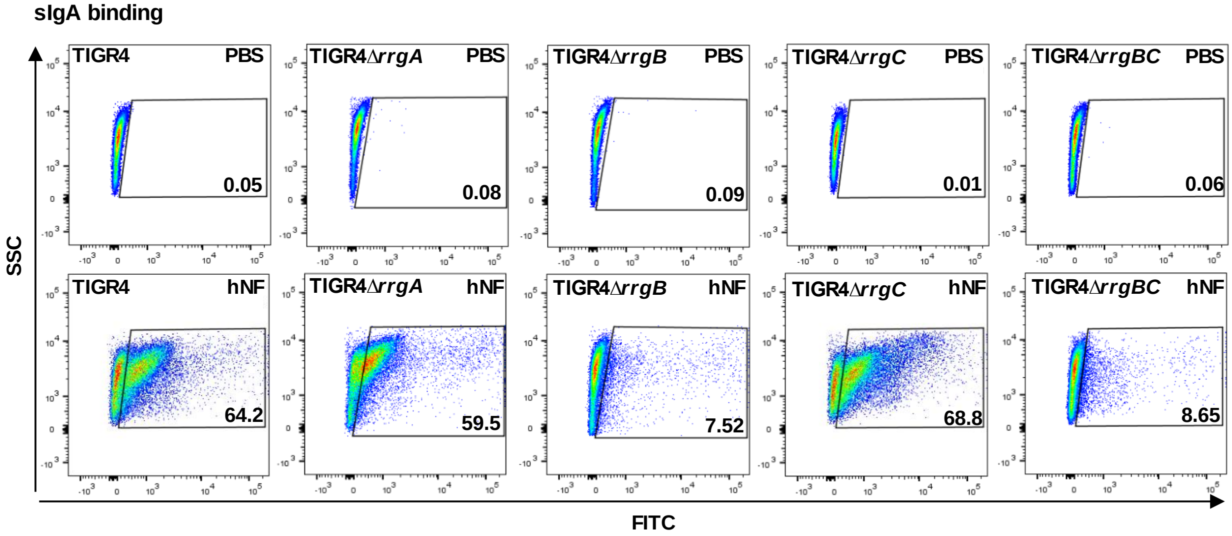


Figure S6: RrgB-mediated binding of mucosal sIgA to *S. pneumoniae*. Representative dot plots showing the percentage acquisition of soluble sIgA from human nasal fluid by Spn mutants deficient in one or two pilus components. Bacteria (5 x 10^6 CFU/ 50 µl) were incubated with 50 µg/ml of hNF. Binding of bacteria-associated sIgA was analyzed by flow cytometry.

Supplemental References

82. Corpet F. Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Res*. 1988;16(22):10881–10890.