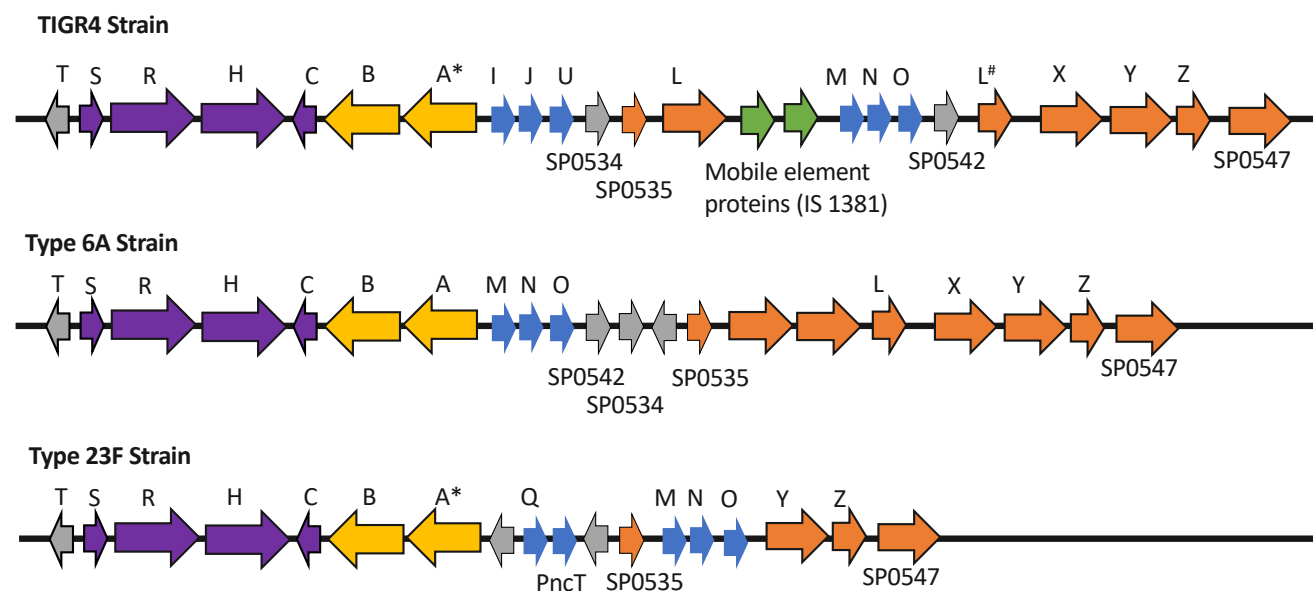
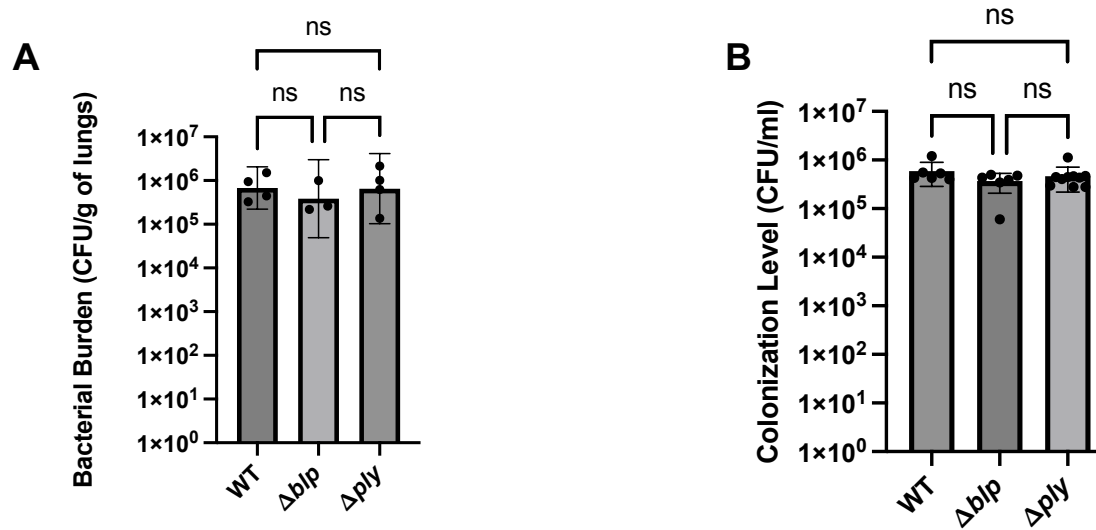


Supplementary Figure 1



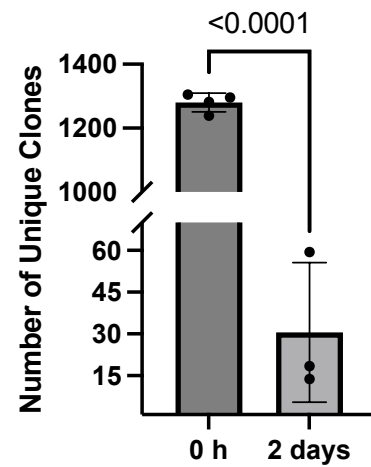
Supplementary Figure 1: Schematic depicting diversity of the *blp* locus. Gene map depicting the *blp* locus in TIGR4, type 6A and type 23F strains. *Purple*: regulatory genes, *yellow*: ABC transporter, *blue*: putative bacteriocins, *orange*: putative immunity and modification proteins, *grey*: hypothetical proteins and proteins of unknown function, and *green*: mobile element proteins. BlpA* denotes non-functional protein due to early truncation. BlpL# denotes protein fragment/pseudogene. The entire region spanning from *blpT* to *sp0547* were deleted in the Δblp strains. Schematic not to scale.

Supplementary Figure 2



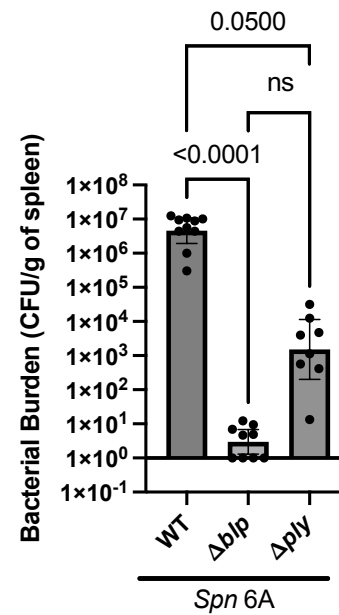
Supplementary Figure 2: Δblp and Δply strains are not inherently defective strains *in vivo*. (A) Bacterial burden of WT, Δblp , and Δply strains in lungs immediately following intranasal inoculation in anesthetized adult mice (0 h). (B) Colonization levels of WT, Δblp , and Δply strains in the URT of infant mice at 3 dpi. *P* values were calculated by Kruskal-Wallis test followed by Dunn's multiple comparisons. 'ns' denotes statistically not significant.

Supplementary Figure 3



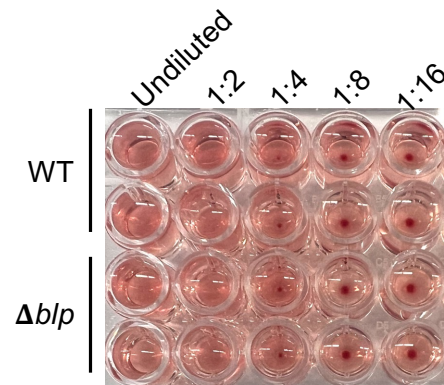
Supplementary Figure 3: WT *Spn* undergoes a loss in diversity during lung infection. Population structure of WT *Spn* in the lungs immediately following (0h) and 2 days post-inoculation, as measured by number of unique clones detected. P-value measured by two-tailed *t*-test.

Supplementary Figure 4



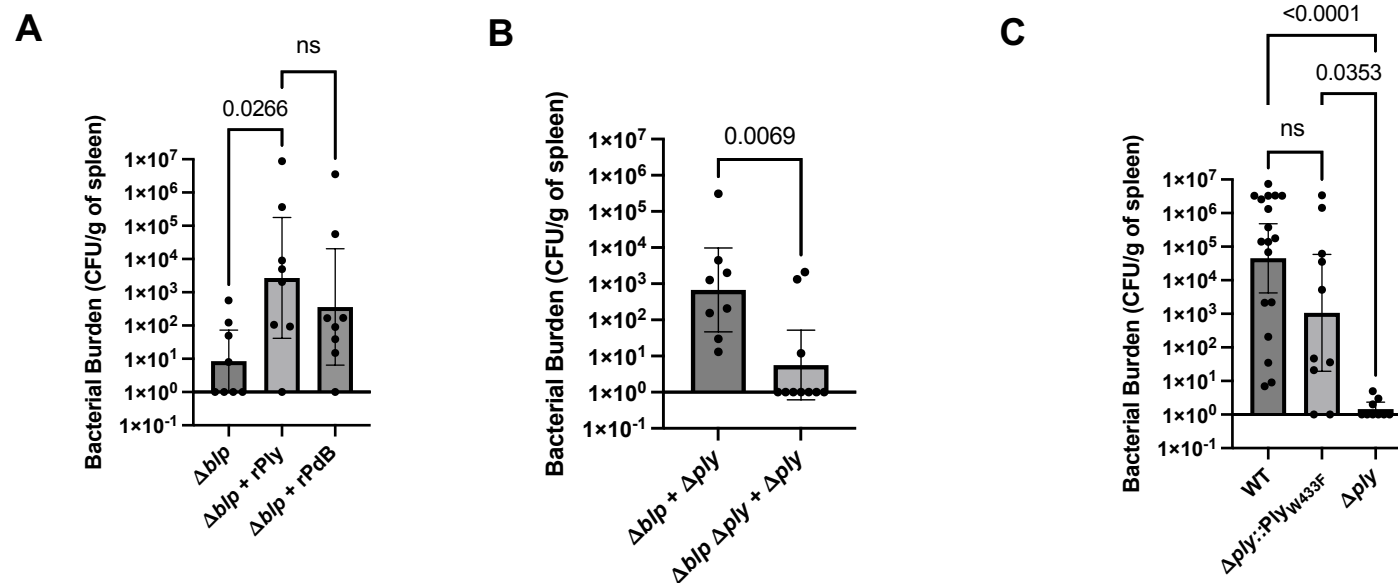
Supplementary Figure 4: Blp bacteriocins promote pneumococcal bacteremia. Bacterial burden in spleens at 2 dpi following 6A *Spn* infection. Each dot represents an individual animal (Geometric mean $\pm$ 95% CI). *P* values were calculated by Kruskal-Wallis test followed by Dunn's multiple comparisons. 'ns' denotes statistically not significant.

Supplementary Figure 5



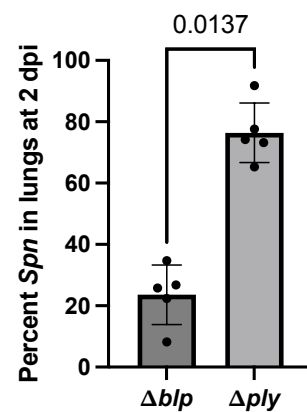
Supplementary Figure 5: Blp bacteriocins do not affect Ply levels. Hemolysis of horse erythrocytes by either WT or Δblp lysates. No difference is observed in the hemolysis levels by lysates of WT and Δblp strains.

Supplementary Figure 6



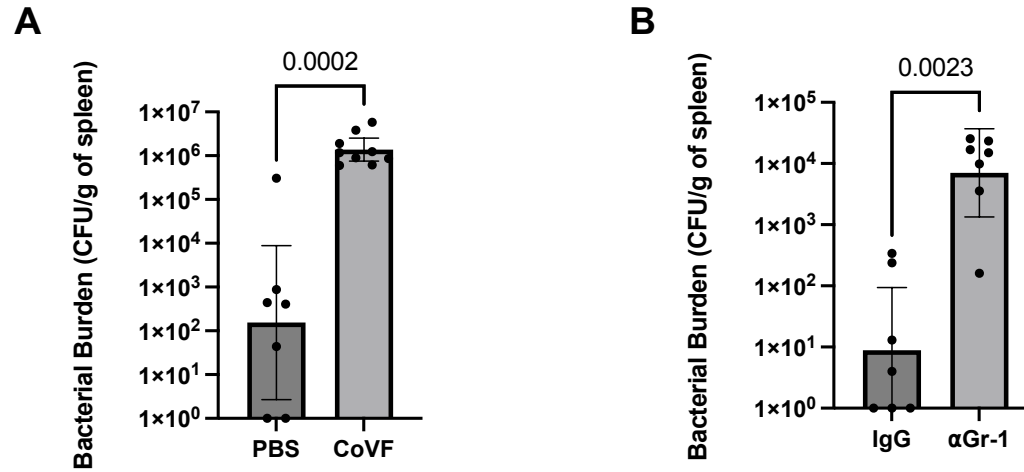
Supplementary Figure 6: Pore-forming activity of Ply is not critical for increased bacterial burden in spleen. Bacterial burden in spleens at 2 dpi following *Spn* infection. **(A)** Complementation of Δbfp with 250ng of recombinant Ply or PdB toxoid increases *Spn* levels in the spleen. **(B)** Animals were inoculated with 1:1 mix of Δply with either Δbfp or $\Delta bfp \Delta ply$ strains. **(C)** W433F point mutation ablates the pore-forming activity of Ply. *P* values were calculated by **(A, C)** Kruskal-Wallis test followed by Dunn's multiple comparisons, or **(B)** Mann-Whitney test. 'ns' denotes statistically not significant.

Supplementary Figure 7



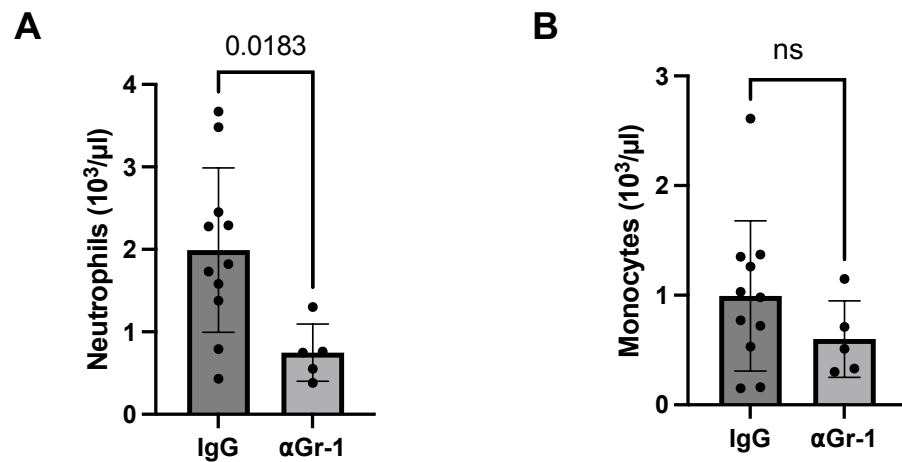
Supplementary Figure 7: Blp bacteriocin-mediated Ply release facilitates bacterial growth in the lungs. Percent bacterial burden in the lungs at 2 dpi following inoculation of 1:1 mix of Δply and Δblp strains. *P* value was calculated by ratio paired *t*-test.

Supplementary Figure 8



Supplementary Figure 8: Inhibition of complement activation results in increased systemic levels of $\Delta b/p$ strain. Bacterial burden in spleens at 2 dpi following $\Delta b/p$ strain infection. (A) Cobra-venom factor (CoVF) treatment, and (B) neutrophil depletion (α Gr-1) result in increased *Spn* levels in the spleen. *P* values were calculated by Mann-Whitney test.

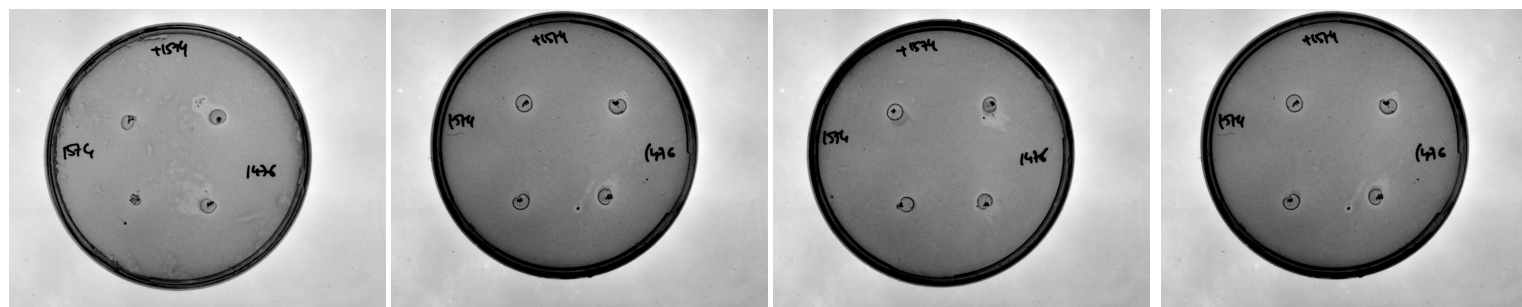
Supplementary Figure 9



Supplementary Figure 9: α Gr-1 results in neutrophil depletion. Numbers of blood (A) neutrophil and (B) monocyte numbers (in $10^3/\mu\text{l}$) from mice at 2 dpi following infection with *Spn*. *P* value calculated by two tailed *t*-test. 'ns' denotes statistically not significant.

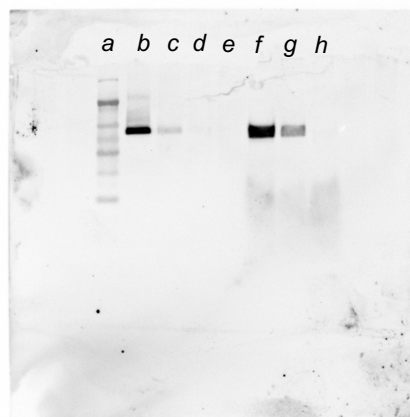
Source Figure associated with Fig 3

A



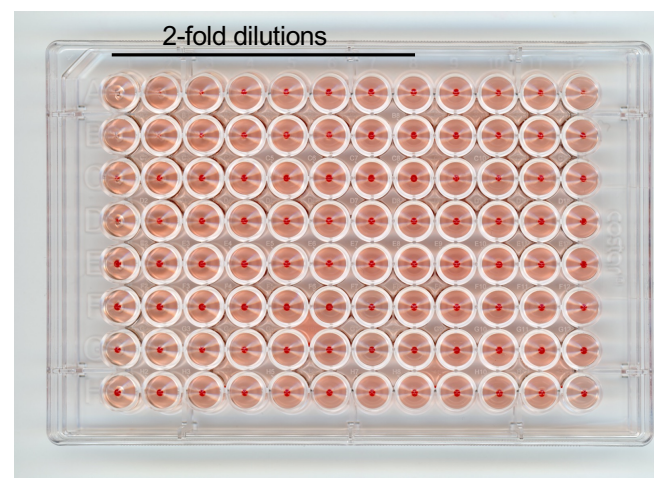
1476: WT
1574: $\Delta blpMNO$

B



Lane a: Ladder
Lane b: rPly (10 ng)
Lane c: rPly (1 ng)
Lane d: rPly (0.1 ng)
Lane e: Empty
Lane f: $\Delta ply + \Delta blp$
Lane g: $\Delta ply\Delta blp + \Delta blp$
Lane h: $\Delta ply + \Delta blp\Delta ply$

C



Rows A-B: $\Delta ply + \Delta blp$
Rows C-D: $\Delta ply\Delta blp + \Delta blp$
Rows E-F: $\Delta ply + \Delta blp\Delta ply$
Rows G-H: $\Delta ply\Delta blp + \Delta blp\Delta ply$

Column 1: Undiluted
Column 8: 1:128 dilution
Columns 9-12: PBS only

Source figure associated with Fig 3. (A) Uncropped images associated with Figs 3A-B. **(B)** Uncropped blot associated with Fig 3C. **(C)** Uncropped image associated with Fig 3D.

Supplementary Table 1: Bacterial strains used in this experimental work

Strain	Strain Number	Description	Reference
TIGR4	P2406	Streptomycin resistant derivative of TIGR4 (T4 WT)	Zafar <i>et al.</i> ⁶⁶
TIGR4 library	-	Molecularly barcoded P2406	This study
<i>blp::Janus</i>	P2702	P2406 with Janus cassette replacing <i>blp</i> locus, kanamycin resistant	This study
Δblp	P2838	P2702 with clean deletion of <i>blp</i> , streptomycin resistant	This study
$\Delta blp::blp$	P2862	P2702 with the <i>blp</i> locus complemented, streptomycin resistant	This study
<i>ply::Janus</i>	P2423	P2406 with Janus cassette replacing <i>ply</i> , kanamycin resistant	Zafar <i>et al.</i> ⁶⁴
Δply	P2851	P2423 with clean deletion of <i>ply</i> , streptomycin resistant	This study
$\Delta ply::Ply_{W433F}$	P2446	P2423 expressing Ply_{W433F} , streptomycin resistant	Zafar <i>et al.</i> ⁶⁴
$\Delta blp \Delta ply$	P2861	P2838 with Janus cassette replacing <i>ply</i> , kanamycin resistant	This study
6A WT	P1476	Streptomycin resistant derivative of a serotype 6A clinical isolate	Dawid <i>et al.</i> ²⁴
$\Delta blpMNO$	P1574	6A WT with a clean deletion of <i>blpMNO</i> , streptomycin resistant	Dawid <i>et al.</i> ²⁴
<i>ply::Janus</i>	P2408	A 23F strain with Janus cassette replacing <i>ply</i> , kanamycin resistant	Kuipers <i>et al.</i> ³²
Δblp	P2706	23F strain with clean deletion of <i>blp</i> , streptomycin resistant	Aggarwal <i>et al.</i> ⁹
$\Delta blp, ply::Janus$	P2758	P2706 with Janus cassette replacing <i>ply</i> , kanamycin resistant	This study
$\Delta blp \Delta ply$	P2759	P2758 with clean deletion of <i>ply</i> , streptomycin resistant	This study
6A Δblp	P2720	P1476 with Janus cassette replacing <i>blp</i> locus, kanamycin resistant	This study
6A <i>ply::Janus</i>	P2435	6A WT with Janus cassette replacing <i>ply</i> , kanamycin resistant	Lokken-Toyli <i>et al.</i> ⁷¹

6A Δply	P2436	P2435 with clean deletion of <i>ply</i> , streptomycin resistant	Lokken-Toyli <i>et al.</i> ⁷¹
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Supplementary Table 2: Primers used in this study

Purpose	Primer Name	Primer Number	Sequence
Construction of <i>blp</i> ::Janus, <i>blp</i> and <i>blp</i> :: <i>blp</i> strains Δ	Blp_Flank1_F	SDA52	ATGAAAGACTTGTTTTTAAAGAGAAAG
	Blp_Flank2_R	SDA57	GATTTGCCTCTAGTAATTCTG
Construction of <i>ply</i> and derivative strains Δ	Ply_Flank1_F	SDA64	CCGAAGCGTTTACGATGATTTCGA
	Ply_Flank2_R	SDA65	TTGCACTATACGCTTAACCAGATTACT
Amplicon Sequencing	IgG_Nested_F	SDA18	GAAAGCGCATCTCAATTTTAAGACT
	IgG_Nested_R	SDA19	CCCTATGTTCTACACCAGTCTCA
	Seq_Adapt_F	SDA30	ACACTCTTTCCCTACACGACGCTCTTCCG ATCTGAGATTTTCAGGTTACACTTATATTGG A
	Seq_Adapt_R	SDA31	GACTGGAGTTCAGACGTGTGCTCTTCCGA TCTTTCGTATGTATTCAAATATATCCTCCT C