SUPPLEMENTAL MATERIAL

The Opp (AmiACDEF) Oligopeptide Transporter Mediates Resistance of Serotype 2 *Streptococcus pneumoniae* D39 to Killing by Chemokine CXCL10 and Other Antimicrobial Peptides

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Table S4. IC_{50} of N-terminal CXCL10-treated *S. pneumoniae* cells with a CFU survival assay in TGS buffer

SUPPLEMENTAL FIGURE LEGENDS.

Fig. S1. *S. pneumoniae* cells in TGS are sensitive to CXCL10 in the 0.03 to 0.06 µM range

Fig. S2. Unencapsulated *S. pneumoniae* D39 show significant decreases in CFUs and low fluorescence after incubation in NPB

Fig. S3. The fluorescence-based antimicrobial assay for IC_{50} determination is not dependent on the basal level of fluorescence signal generated by untreated bacteria

Fig. S4. The Δ*amiA-F* clean deletion mutant phenocopies the Δ*amiA-F*::P_c-*aad9* strain, and the Δ*amiA-F* mutation is responsible for the phenotype seen in the ΔOPT mutant, and CXCL10 killing of the Δ*amiA-F* strain is consistent with both fluorescence-based and CFU survival assays

Fig. S5. *Spn* D39 Δ*amiA-F* mutants in NPB are more resistant to nisin, whereas *Spn* D39 Δ*dlt* and *Spn* TIGR4 are more sensitive to LL-37 and nisin relative to *Spn* D39

Fig. S6. Sensitivity to CXCL10 and dose response curves of *S. mitis*, *S. sanguinis*, and *S. mutans* to LL-37

Fig. S7. Δ*ftsX* and Δ*ftsE* mutants of *B. subtilis* show similar IC_{50} values to CXCL10 or N-CXCL10 as the *ftsE*$^+$ *ftsX*$^+$ parent

Fig. S8. CXCL10 does not show antimicrobial activity against *Spn* D39 in DMEM ± 10% (vol/vol) FBS

SUPPLEMENTAL REFERENCES

Table S1. Bacterial strains and oligonucleotide primers used in this study

***Streptococcus pneumoniae* strains used in this study**			
Strain Number	Genotype (description)	Antibiotic resistance[a]	Reference or source
IU1686	TIGR4 [JNR.7/87]	None	ATCC
IU1690	D39	None	(1)
IU1781	D39 *rpsL1*	Str^R	(2)
IU1945	D39 Δ*cps2A'-cps2H'*= D39 ∆*cps*	None	(1)
IU3309	D39 *rpsL1 cps2E* (ΔA); *cps2E* has a ΔA frameshift at codon 326	Str^R	(2)
IU4325	D39 Δ*cps rpsL1 ftsEX*$^+$ P_c-[*kan-rpsL*$^+$]	Kan^R, Str^S	(3)
IU6220	D39 Δ*cps ftsX*(S161Y) P_c-[*kan-rpsL*$^+$] (IU1945 X *ftsEX* P_c-[*kan-rpsL*$^+$] error-prone amplicon)	Kan^R	This study
IU6234	D39 Δ*cps ftsX*(D129V) P_c-[*kan-rpsL*$^+$] (IU1945 X *ftsEX* P_c-[*kan-rpsL*$^+$] error-prone amplicon)	Kan^R	This study
IU6236	D39 Δ*cps ftsX*(N269T) P_c-[*kan-rpsL*$^+$] (IU1945 X *ftsEX* P_c-[*kan-rpsL*$^+$] error-prone amplicon)	Kan^R	This study
IU6237	D39 Δ*cps ftsX*(F45I, K95M, E117K, V139A, E213V) P_c-[*kan-rpsL*$^+$] (IU1945 X *ftsEX* P_c-[*kan-rpsL*$^+$] error-prone amplicon)	Kan^R	This study
IU6321	D39 Δ*cps pcsB*(A160P) P_c-*erm ftsX*(W183L) P_c-[*kan-rpsL*$^+$]	Kan^R, Erm^R	(3)
IU8773	D39 *ftsX*(S161Y) P_c-[*kan-rpsL*$^+$] (IU1690 X *ftsX* (S161Y) P_c-[*kan-rpsL*$^+$] from IU6220)	Kan^R	This study
IU9004	D39 *ftsX*(N269T) P_c-[*kan-rpsL*$^+$] (IU1690 X *ftsX* (N269T) P_c-[*kan-rpsL*$^+$] from IU6236)	Kan^R	This study
IU9008	D39 *ftsX*(W183L) P_c-[*kan-rpsL*$^+$] (IU1690 X *ftsX*(W183L) P_c-[*kan-rpsL*$^+$] from IU6321)	Kan^R	This study
IU9110	D39 *ftsX*(D129V) P_c-[*kan-rpsL*$^+$] (IU1690 X *ftsX* (D129V) P_c-[*kan-rpsL*$^+$] from IU6234)	Kan^R	This study
IU9113	D39 *ftsX*(E213V) P_c-[*kan-rpsL*$^+$] (IU1690 X *ftsX*(E213V) P_c-[*kan-rpsL*$^+$] from IU6237)	Kan^R	This study
IU9621	D39 Δ*cps rpsL1* Δ*khpA* // Δ*bgaA*::*kan* t1t2 P_{ftsA}-*khpA*$^+$ (IU9036 X fusion Δ*bgaA*::*kan* t1t2 P_{ftsA}-*khpA*$^+$)	Str^R, Kan^R	(4)
IU10748	D39 *ftsX* P_c-[*kan-rpsL*$^+$] (IU1690 X *ftsX*$^+$ P_c-[*kan-rpsL*$^+$] from IU4325)	Kan^R	This study
IU11720	D39 Δ*amiACDEF*::P_c-[*kan-rpsL*$^+$] (IU1690 X fusion Δ*amiACDEF*::P_c-[*kan-rpsL*$^+$])	Kan^R	This study
IU11759	D39 Δ*amiACDEF*::P_c-*aad9* (IU1690 X fusion Δ*amiACDEF*::P_c-*aad9*) = Δ*amiA-F*	Spc^R	This study
IU11778	D39 Δ[*spd_1167-spd_1170*]::P_c-*cat* (IU1690 X fusion Δ[*spd_1167-spd_1170*]::P_c-*cat*)	Cat^R	This study
IU11819	TIGR4 Δ[*cps4A'-cps4E'*]::P_c-*cat* (IU1686 X fusion Δ[*cps4A-cps4E*']:P_c-*cat*)	Cat^R	This study

IU11848	D39 Δ*aliA*::P_c-[*kan-rpsL*$^+$] (IU1690 X Δ*aliA*::P_c-[*kan-rpsL*$^+$] from K218)	Kan^R	This study
IU11850	D39 Δ*aliB*::P_c-*erm* (IU1690 X Δ*aliB*::P_c-*erm* from E241)	Erm^R	This study
IU11867	D39 Δ*amiACDEF*::P_c-*aad9* Δ[*spd_1167-spd_1170*]::P_c-*cat* (IU11759 X Δ[*spd_1167-spd_1170*]::P_c-*cat* from IU11778)	Spc^R, Cat^R	This study
IU11892	D39 Δ*amiACDEF*::P_c-*aad9* Δ[*spd_1167-spd_1170*]::P_c-*cat* Δ*aliA*::P_c-[*kan-rpsL*$^+$] (IU11867 X Δ*aliA*::P_c-[*kan-rpsL*$^+$] from IU11848)	Spc^R, Cat^R, Kan^R	This study
IU11919	D39 Δ*amiACDEF*::P_c-*aad9* Δ[*spd_1167-spd_1170*]::P_c-*cat* Δ*aliA*::P_c-[*kan-rpsL*$^+$] Δ*aliB*::P_c-*erm* (IU11892 X Δ*aliB*::P_c-*erm* from IU11850) = ΔOPT	Spc^R, Cat^R, Kan^R, Erm^R	This study
IU11966	TIGR4 P1542	None	(5, 6)
IU12001	TIGR4 Δ[*cps4A'-cps4E'*]::P_c-*cat* = TIGR4 Δ*cps* (IU11966 X Δ[*cps4A-cps4E*]::P_c-*cat* from IU11819)	Cat^R	This study
IU12163	D39 Δ*cls*::P_c-*erm* (IU1690 X Δ*cls*::P_c-*erm* from E422)	Erm^R	This study
IU12470	D39 Δ*dltA*::P_c-*erm* (IU1690 X fusion Δ*dltA*::P_c-*erm*)	Erm^R	This study
IU13765	D39 *rpsL1* Δ*amiACDEF*::P_c-[*kan-rpsL*$^+$] (IU1781 X Δ*amiACDEF*::P_c-[*kan-rpsL*$^+$] from IU11720)	Kan^R	This study
IU13780	D39 *rpsL1* Δ*amiACDEF* (IU13765 X fusion Δ*amiACDEF*)	Str^R	This study
IU14488	D39 Δ*amiD* (IU1690 X Δ*amiD* from E120)	Erm^R	This study
IU14510	D39 Δ*amiD* // Δ*bgaA*::*kan* t1t2 P_{ftsA}-*amiD*$^+$ (IU14488 X fusion Δ*bgaA*::*kan* t1t2 P_{ftsA}-*amiD*$^+$)	Kan^R	This study
E120	D39 Δ*cps* Δ*amiD*::P_c-*erm* (IU1945 X fusion Δ*amiD*::P_c-*erm*)	Erm^R	This study
E241	D39 Δ*cps* Δ*aliB*::P_c-*erm* (IU1945 X fusion Δ*aliB*::P_c-*erm*)	Erm^R	This study
E422	D39 Δ*cps* Δ*cls*::P_c-*erm* (IU1945 X fusion Δ*cls*::P_c-*erm*)	Erm^R	This study
K218	D39 Δ*cps* Δ*aliA*::P_c-[*kan-rpsL*$^+$] (IU1945 X fusion Δ*aliA*::P_c-[*kan-rpsL*$^+$])	Kan^R	This study
Other *Streptococcus* strains used in this study			
Strain number	Genotype (description)	Antibiotic resistance[a]	Reference or source
IU11303	*S. mitis* ATCC 49456; NCTC 12261 [NS 51]	None	ATCC
IU11305	*S. sanguinis* ATCC 10556; DSS-10	None	ATCC

IU11309	*S. mutans* ATCC 25175; NCTC 10449 [IFO 13955]	None	ATCC
Bacillus subtilis strains used in this study			
Strain number	Genotype (description)	Antibiotic resistance[a]	Reference or source
IU12153	DK453, ancestral NCIB3610 Δ*spoIIIE*::*kan*	Kan^R	(7)
IU12165	NCIB3610 Δ*spoIIIE*::*kan* Δ*ftsX*::Tn10 *spec*	Kan^R, Spc^R	Gift of D. Kearns
IU12166	NCIB3610 Δ*spoIIIE*::*kan* Δ*ftsE*::Tn10 *spec*	Kan^R, Spc^R	
IU12981	NCIB3610 Δ*spoIIIE*::*kan* Δ*ftsX*	Kan^R	
Primers used to construct strains			
Primer	Sequence (5' to 3')	Template[b]	Amplicon Product
For construction of IU6220, IU6234, IU6236, IU6237 using error-prone PCR			
CS235	CACCTCTGTTATTTTCAATACAGCGAAACT AGCTAC	IU4325	*ftsEX*$^+$ P_c-[*kan-rpsL*$^+$] error-prone amplicon; *ftsEX*$^+$ to 979 bp downstream of P_c-[*kan-rpsL*$^+$]
CS129	GGTGAAGACCAAATGGCAAGAGCAAACG		
For construction of IU8773 (*ftsX*(S161Y) P_c-[*kan-rpsL*$^+$])			
CS128	TAAATACCTTGCGCCACCGTGTCATTGC	IU6220	3' *ftsE* + *ftsX*(S161Y) P_c-[*kan-rpsL*$^+$]
CS129	GGTGAAGACCAAATGGCAAGAGCAAACG		
For construction of IU9004 (*ftsX*(N269T) P_c-[*kan-rpsL*$^+$])			
CS128	TAAATACCTTGCGCCACCGTGTCATTGC	IU6236	3' *ftsE* + *ftsX* (N269T) P_c-[*kan-rpsL*$^+$]
CS129	GGTGAAGACCAAATGGCAAGAGCAAACG		
For construction of IU9008 (*ftsX*(W183L) P_c-[*kan-rpsL*$^+$])			
CS128	TAAATACCTTGCGCCACCGTGTCATTGC	IU6321	3' *ftsE* + *ftsX* (W183L) P_c-[*kan-rpsL*$^+$]
CS129	GGTGAAGACCAAATGGCAAGAGCAAACG		
For construction of IU9110 (*ftsX*(D129V) P_c-[*kan-rpsL*$^+$])			
CS128	TAAATACCTTGCGCCACCGTGTCATTGC	IU6234	3' *ftsE* + *ftsX* (D129V) P_c-[*kan-rpsL*$^+$]
CS129	GGTGAAGACCAAATGGCAAGAGCAAACG		
For construction of IU9113 (*ftsX*(E213V) P_c-[*kan-rpsL*$^+$])			
CS128	TAAATACCTTGCGCCACCGTGTCATTGC	IU6237	3' *ftsE* +

CS129	GGTGAAGACCAAATGGCAAGAGCAAACG		*ftsX*(E213V) P_c-[*kan-rpsL*$^+$]
For construction of IU10748 (*ftsX*$^+$ P_c-[*kan-rpsL*$^+$])			
CS128	TAAATACCTTGCGCCACCGTGTCATTGC	IU4325	3' *ftsE* + *ftsX*$^+$ P_c-[*kan-rpsL*$^+$]
CS129	GGTGAAGACCAAATGGCAAGAGCAAACG		
For construction of IU11720 (Δ*amiACDEF*::P_c-[*kan-rpsL*$^+$])			
P25	TGCTCCTGTTCGCTTGATGATGGA	D39	5' upstream of *amiA* + 60 bp of 5' *amiA*
P29	CATTATCCATTAAAAATCAAACGGATCCTAAAGTACACCTGCTGCTAATAAAACAAGACC		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-[*kan-rpsL*$^+$] cassette[c]	P_c-[*kan-rpsL*$^+$]
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
KB411	CAAAAGCATAAGGAAAGGGGCCCGGTCACTATGTTTGGGCGAACCAAGCCGAA	D39	57 bp of 3' *amiF* + 3' downstream of *amiF*
KB412	CTTCGCTACGATAGAGTTGTCCGATGTCGC		
For construction of IU11759 (Δ*amiACDEF*::P_c-*aad9*)			
P25	TGCTCCTGTTCGCTTGATGATGGA	D39	5' upstream of *amiA* + 60 bp of 5' *amiA*
P29	CATTATCCATTAAAAATCAAACGGATCCTAAAGTACACCTGCTGCTAATAAAACAAGACC		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*aad9* cassette from IU8429[d]	P_c-*aad9*
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
KB411	CAAAAGCATAAGGAAAGGGGCCCGGTCACTATGTTTGGGCGAACCAAGCCGAA	D39	57 bp of 3' *amiF* + 3' downstream of *amiF*
KB412	CTTCGCTACGATAGAGTTGTCCGATGTCGC		
For construction of IU11778 (Δ[*spd_1167-spd_1170*]::P_c-*cat*)			
P1043	CCAGGATTAGCTGGGATGATTTGTAGACGG	D39	5' upstream of *spd_1170* + 75 bp of 5' *spd_1170*
P1045	CATTATCCATTAAAAATCAAACGGATCCTAACTACAAGCAACCAATCCTACTACAGCTAT		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*cat* cassette[e]	P_c-*cat*
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
KB413	AAACGTCCAAAAGCATAAGGAAAGGGGCCCGTTGAATTTAAAGCACAGTACCATGAATTT	D39	102 bp of 3' *spd_1167* + 3'

KB414	ATCAGCACTCGTAGATACACACGGCAAGC		downstream of *spd_1167*
For construction of IU11819 (TIGR4 Δ[*cps4A'-cps4E'*]::P_c-*cat*)			
KB419	CATTATCCATTAAAAATCAAACGGATCCTA CGACACCGAACTAATAGGACCATAGGTG	TIGR4 (IU1686)	5' upstream of *cps4A* + 5' 51 bp of *cps4A*
KB420	CACGTTCACAGAAAGTGAAGCGAAGTG		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*cat* cassette[e]	P_c-*cat*
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
KB416	CCTCCAAAGAACGTCTTCCATAGAAGG	TIGR4 (IU1686)	42 bp of 3' *cps4E* + 3' downstream of *cps4E*
KB418	AAACGTCCAAAAGCATAAGGAAAGGGGCC CAAACGAAAAGGTATTGTAGAGGGTAGTG GT		
For construction of IU11848 (D39 Δ*aliA*::P_c-[*kan-rpsL*$^+$])			
P527	AGTCCAAAGTTTAGGAGCAAGGCGACGCT A	K218	Δ*aliA*::P_c-[*kan-rpsL*$^+$]
P528	TTTCCATTGGCATCAACGGTCAAGCCCTTC		
For construction of IU11850 (D39 Δ*aliB*::P_c-*erm*)			
P555	TTCTTGCTACCAGCAACGGTTGGAGTGGTT	E241	Δ*aliB*::P_c-*erm*
P556	GCCGCAAAGATAAATAAGAGAGCAAACGA GGTCT		
For construction of IU11867 (D39 Δ*amiACDEF*::P_c-*aad9* Δ[*spd_1167-spd_1170*]::P_c-*cat*)			
P1043	CCAGGATTAGCTGGGATGATTTGTAGACG G	IU11778	Δ[*spd_1167-spd_1171*]::P_c-*cat*
KB414	ATCAGCACTCGTAGATACACACGGCAAGC		
For construction of IU11892 (D39 Δ*amiACDEF*::P_c-*aad9* Δ[*spd_1167-spd_1170*]::P_c-*cat* Δ*aliA*::P_c-[*kan-rpsL*$^+$])			
P527	AGTCCAAAGTTTAGGAGCAAGGCGACGCT A	IU11848	Δ*aliA*::P_c-[*kan-rpsL*$^+$]
P528	TTTCCATTGGCATCAACGGTCAAGCCCTTC		
For construction of IU11919 (D39 Δ*amiACDEF*::P_c-*aad9* Δ[*spd_1167-spd_1170*]::P_c-*cat* Δ*aliA*::P_c-[*kan-rpsL*$^+$] Δ*aliB*::P_c-*erm*)			
P555	TTCTTGCTACCAGCAACGGTTGGAGTGGTT	IU11850	Δ*aliB*::P_c-*erm*
P556	GCCGCAAAGATAAATAAGAGAGCAAACGA GGTCT		
For construction of IU12001 (TIGR4 Δ[*cps4A'-cps4E'*]::P_c-*cat*)			
KB420	CACGTTCACAGAAAGTGAAGCGAAGTG	IU11819	Δ[*cps4A'-cps4E'*]::P_c-*cat*
KB416	CCTCCAAAGAACGTCTTCCATAGAAGG		
For construction of IU12163 (D39 Δ*cls*::P_c-*erm*)			
P943	TCCCTGCCTTGACTCGCTTGGTTGAGTTTA	E422	Δ*cls*::P_c-

P944	ATGTCCAGCTTGGTCTCCTTGCTCTGTCAA		*erm*
For construction of IU12470 (D39 Δ*dltA*::P_c-*erm*)			
P1659	CAAAGGTTGGAAGTTAGTTGCTAGAAATCC	D39	5’ upstream of *dltA* + 5’ 84 bp of *dltA*
P1661	CATTATCCATTAAAAATCAAACGGATCCTAA ACATTATAGACAGGATAGCTAGGCTGTGT		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[c]	P_c-*erm*
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1662	AAACGTCCAAAAGCATAAGGAAAGGGGCC CACTCCAAATGGAAAGATTGACATCAAAGG A	D39	3’ 54 bp of *dltA* + 3’ downstream of *dltA*
P1660	CGATATAGTACCAGGTCACACCATGCC		
For construction of IU13765 (D39 *rpsL1* Δ*amiACDEF*::P_c-[*kan-rpsL*$^+$])			
P25	TGCTCCTGTTCGCTTGATGATGGA	IU11720	Δ*amiA-F*::P_c-[*kan-rpsL*$^+$]
KB412	CTTCGCTACGATAGAGTTGTCCGATGTCGC		
For construction of IU13780 (D39 *rpsL1* Δ*amiACDEF*)			
P25	TGCTCCTGTTCGCTTGATGATGGA	D39	5’ upstream of *amiA* + 5’ 60 bp of *amiA*
KB430	GGTTCGCCCAAACATAGTGACCAAGTACA CCTGCTGCTAATAAAACAAGACCTGC		
KB429	CTACAGCAGGTCTTGTTTTATTAGCAGCAG GTGTACTTGGTCACTATGTTTGGGCGAACC	D39	3’ 57 bp of *amiF* + 3’ downstream of *amiF*
KB412	CTTCGCTACGATAGAGTTGTCCGATGTCGC		
For construction of IU14510 (D39 Δ*amiD* *ll* *bgaA*::*kan*-T1T2-P_{ftsA}-*amiD*$^+$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9621	3’ PTS EII + P_c-*kan* + t1t2 + P_{ftsA} + 5’ 13 bp of *amiD*
KB448	CGATTGTAGACATTACATCGCTTCCTCTCT ATCTTCCAAGTTTCG		
KB447	ATAGAGAGGAAGCGATGTAATGTCTACAAT CGATAAAGAAAAATTTCAGTTTGTAAAACG	D39	3’ 19 bp of P_{ftsA} + *amiD* + 34 bp of 3’ *bgaA*
KB446	CCGCAGCAACTGGTTTATGAGAAAGTAAGT TCTTCTATCTATGTGTACGTGGATCACTAG		
KB445	CTAGTGATCCACGTACACATAGATAGAAGA ACTTACTTTCTCATAAACCAGTTGCTGCGG	IU9621	3’ 26 bp of *amiD* + 3’ *bgaA*
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of E120 (D39 Δ*cps* Δ*amiD*::P_c-*erm*)			
P380	CTCAAGAACCTACAAGTCACCTAGTCAGGC	D39	5’ upstream of *amiD* + 5’ 60 bp of *amiD*
P382	CATTATCCATTAAAAATCAAACGGATCCTAT TCAGAGGCAAAATCGTCACGTTTTACAAA		

kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[c]	P_c-*erm*
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P383	CAAAAGCATAAGGAAAGGGGCCCTCCCTTTTCGTAGTTGGTCAAAACTTAG	D39	3’ 60 bp of *amiD* + 3’ dowstream of *amiD*
P381	CCCTTTCAGGTCAGTATAAAGTGACGGAGG		
For construction of E241 (D39 Δ*cps* Δ*aliB*::P_c-*erm*)			
P555	TTCTTGCTACCAGCAACGGTTGGAGTGGTT	D39	5’ upstream of *aliB* + 5’ 60 bp of *aliB*
P557	CATTATCCATTAAAAATCAAACGGATCCTAAACTCCTGTACCCAGGACAAGACCTG		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[c]	P_c-*erm*
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P558	CAAAAGCATAAGGAAAGGGGCCCAAAGAAAAAGAAGAATCCAATAAAAAAGCCC	D39	3’ 57 bp of *aliB* + 3’ dowstream of *aliB*
P556	GCCGCAAAGATAAATAAGAGAGCAAACGAGGTCT		
For construction of E422 (D39 Δ*cps* Δ*cls*::P_c-*erm*)			
P943	TCCCTGCCTTGACTCGCTTGGTTGAGTTTA	D39	5’ upstream of *cls* + 5’ 60 bp of *cls*
P945	CATTATCCATTAAAAATCAAACGGATCCTACATAATCGAAAGACTAAAGCCATACTTGG		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[c]	P_c-*erm*
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P946	CAAAAGCATAAGGAAAGGGGCCCTCTCAGGAAGTCTATCCTCATTCTATCA	D39	3’ 87 bp of *cls* + 3’ downstream of *cls*
P944	ATGTCCAGCTTGGTCTCCTTGCTCTGTCAA		
For construction of K218 (D39 Δ*cps* Δ*aliA*::P_c-[*kan-rpsL*$^+$])			
P527	AGTCCAAAGTTTAGGAGCAAGGCGACGCTA	D39	5’ upstream of *aliA* + 5’ 60 bp of *aliA*
P529	CATTATCCATTAAAAATCAAACGGATCCTATAAAGTAGTCGCCGCCAATAATGTCAC		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-[*kan-rpsL*$^+$] cassette[c]	P_c-[*kan-rpsL*$^+$]
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P530	CAAAAGCATAAGGAAAGGGGCCCCACTGTAGATGAATACCAAAAAGCTCAGGA	D39	3’ 100 bp of *aliA* + 3’

P528	TTTCCATTGGCATCAACGGTCAAGCCCTTC		downstream of *aliA*

[a]Antibiotic resistance markers: Erm^R, erythromycin; Kan^R, kanamycin; $Spec^R$, spectinomycin; Str^R, streptomycin; Cat^R, chloramphenicol.

[b]Genomic DNA of indicated *S. pneumoniae* strains was used as templates for PCR reactions, except for P_c-[*kan-rpsL*$^+$], P_c-*erm*, P_c-*cat*, and P_c-*aad9* cassettes.

[c]P_c-[*kan-rpsL*$^+$], and P_c-*erm* are described in (8, 9).

[d]The P_c-*aad9* cassette was amplified from IU8429 (10).

[e]P_c-*cat* cassette was obtained from IU10294 (9).

Table S2. IC_{50} of *S. pneumoniae* D39 cells treated with CXCL10, LL-37, or nisin under various conditions[a]

	CXCL10		LL-37		Nisin	
	IC_{50} (μM)[b]	95% CI (μM)[c]	IC_{50} (μM)[b]	95% CI (μM)[c]	IC_{50} (μM)[b]	95% CI (μM)[c]
1. TGS-CFU	0.016	0.015–0.017	0.063	0.057–0.070	0.25	0.19–0.32
2. NPB-CFU	1.9	1.5–2.4	0.67	0.47–0.95	0.76	0.70–0.83
3. NPB-Fluor	1.7	1.1–2.7	1.6	1.5–1.7	0.80	0.71–0.90

[a] IC_{50} and 95% CI of *Spn* D39 to CXCL10, LL-37, and nisin were determined using a CFU survival assay in TGS buffer (10 mM Tris-HCl, pH 7.4 with 5 mM glucose) (TGS-CFU), or in NPB buffer (10 mM sodium phosphate, pH 7.4 with 1% (vol/vol) BHI broth) (NPB CFU), or with a fluorescence-based antimicrobial assay in NPB (NPB-Fluor) as described in Materials and Methods. Inhibition curves from which IC_{50} and 95% CI values were obtained are shown in Fig. 2.

[b] IC_{50} values were obtained from pooled data from at least two independent experiments and fit to a dose-response curve (log of inhibitor vs. response-variable slope) using GraphPad Prism.

[c] 95% confidence interval of the IC_{50} value.

Table S3. IC_{50} of chemokine-treated *Bacillus subtilis* under various conditions

B. subtilis strains[a]	CXCL10					N-terminal CXCL10	
	NPB[b]		DMEM + 10% (vol/vol) FBS[c]	TGS[d]		TGS[d]	
	IC_{50} (μM)[e]	95% CI (μM)[f]	IC_{50} (μM)	IC_{50} (μM)[e]	95% CI (μM)[f]	IC_{50} (μM)[e]	95% CI (μM)[f]
1. *ftsX*$^+$ *ftsE*$^+$ parent	0.69	0.67–0.71	>5.8[g]	0.51	0.36–0.72	0.38	0.33–0.43
2. Δ*ftsX*	0.73	0.60–0.90	>5.8[g]	0.42	0.32–0.55	0.52	0.40–0.67
3. Δ*ftsE*	0.73	0.56–0.96	nd[h]	nd[h]		0.43	0.27–0.68

[a] *B. subtilis* strains used were *ftsX*$^+$ *ftsE*$^+$ parent (IU12153), Δ*ftsX* (IU12981) for assays in NPB and DMEM +10% (vol/vol) FBS, Δ*ftsX*::Tn10 *spec* (IU12165) for assays in TGS, and Δ*ftsE*::Tn10 *spec* (IU12166).

[b] IC_{50} and 95% CI were determined using a fluorescence-based antimicrobial assay in NPB as described in Materials and Methods. Inhibition curves from which IC_{50} and 95% CI values were obtained are shown in Fig. 6. A CFU survival assay was performed under the same conditions and results are shown in Fig. S7A.

[c] IC_{50} values were estimated from a fluorescence-based antimicrobial assay and a CFU survival assay in DMEM +10% (vol/vol) FBS as described in Materials and Methods. Graphs from which IC_{50} values were estimated are shown in Fig. S7D and S7E.

[d] IC_{50} and 95% CI were determined using a CFU survival assay in TGS as described in Materials and Methods. Inhibition curves from which IC_{50} and 95% CI values were obtained are shown in Fig. S7B and S7C.

[e] IC_{50} values were obtained from pooled data from at least two independent experiments and fit to a dose-response curve (log of inhibitor vs. response-variable slope) using GraphPad Prism.

[f] 95% confidence interval of the IC_{50} value.

[g] Less than 50% inhibition was obtained with both the fluorescence-based antimicrobial assay and the CFU survival assay at the highest concentration (5.8 µM) of CXCL10 (see Fig. S7D and S7E).

[h] nd, not determined.

Table S4. IC_{50} of N-terminal CXCL10-treated *S. pneumoniae* cells with a CFU survival assay in TGS buffer[a]

	N-terminal CXCL10	
S. pneumoniae strains[b]	IC_{50} (μM)[c]	95% CI[d]
1. D39	0.14	0.12–0.16
2. *ftsX*$^+$ P_c-[*kan-rpsL*$^+$]	0.14	0.12–0.16
3. *ftsX* (W183L) P_c-[*kan-rpsL*$^+$]	0.15	0.12–0.19
4. *ftsX* (N269T) P_c-[*kan-rpsL*$^+$]	0.13	0.10–0.18
5. *ftsX* (S161Y) P_c-[*kan-rpsL*$^+$]	0.12	0.11–0.13
6. *ftsX* (D129V) P_c-[*kan-rpsL*$^+$]	0.17	0.11–0.27
7. *ftsX* (E213V) P_c-[*kan-rpsL*$^+$]	0.15	0.12–0.19

[a] IC_{50} and 95% CI were determined using a CFU survival assay in TGS as described in Materials and Methods.

[b] Strains used are in D39 genetic background and are as follows: D39, IU1690; *ftsX*$^+$ P_c-[*kan-rpsL*$^+$], IU10748; *ftsX* (W183L) P_c-[*kan-rpsL*$^+$], IU9008; *ftsX* (N269T) P_c-[*kan-rpsL*$^+$], IU9004; *ftsX* (S161Y) P_c-[*kan-rpsL*$^+$], IU8773; *ftsX* (D129V) P_c-[*kan-rpsL*$^+$], IU9110; *ftsX* (E213V) P_c-[*kan-rpsL*$^+$], IU9113. The range of N-terminal CXCL10 concentrations used was 0.014 to 1.85 μM for IU1690 and 0.014 to 0.463 μM for all other strains

[c] IC_{50} values were obtained from pooled data from at least two independent experiments and fit to a dose-response curve (log of inhibitor vs. response-variable slope) using GraphPad Prism.

[d] 95% confidence interval of the IC_{50} value.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. *S. pneumoniae* cells in TGS are sensitive to CXCL10 in the 0.03 to 0.06 µM range. A CFU survival assay in TGS (10 mM Tris-HCl 5 mM glucose, pH 7.4) in the presence of CXCL10 was performed as described in Materials and Methods. D39 (IU1690, black) and D39 Δ*cps* (IU1945, red) are encapsulated and unencapsulated strains of serotype 2 *Spn* respectively, and TIGR4 (IU11966, blue), and TIGR4 Δ*cps* (IU12001, green) are encapsulated and unencapsulated strains of serotype 4 *Spn*, respectively. n indicates the number of independent experiments, each containing duplicate reactions. Each data point represents the mean ± SEM (if not visible, error bars are smaller than the symbol). Dose-response curves were fit to pooled data in GraphPad Prism, using the (log of inhibitor vs. response-variable slope) function. See Table 1 for 95% CI values.

Fig. S2. Unencapsulated *S. pneumoniae* D39 show significant decreases in CFUs and low fluorescence after incubation in NPB. (A) Percentage CFU survival after 2 h of incubation in TGS (10 mM Tris-HCl with 5 mM glucose, pH 7.4; left two columns) or NPB (10 mM sodium phosphate, pH 7.4 with 1% (vol/vol) BHI broth; right four columns). Strains from left to right are D39 *cps*$^+$ (IU1690), D39 Δ*cps* (IU1945), D39 *cps*$^+$ (IU1690), D39 Δ*cps* (IU1945), D39 *rpsL1 cps*$^+$ (IU1781) and D39 *rpsL1 cps2E* (ΔA) (IU3309) containing a frameshift mutation within *cps2E.* Strains were grown in BHI broth and resuspended in the indicated buffers as described in Materials and Methods. Cells were serially diluted and plated for CFU both before and after incubation, and % survival (CFU_{2h}/CFU_{0h}) ± standard error (SE) for each strain is shown. n=2, except for the following: D39 in TGS, n=5; D39 Δ*cps* in TGS, n=11; D39 in NPB, n=7. Statistical

significance was determined using Mann-Whitney T-test. ns, not significant; *, $p < 0.05$; **, $p < 0.01$. (B) Representative plots of fluorescence over time for incubations of strains in NPB. Resazurin dye was added after 2 h to cell suspensions in NPB and fluorescence was measured until approximately 5 h. Numbers to the right of each plot show the raw fluorescence value in Arbitrary Fluorescence Units (AFU), as well as the percent relative to *Spn* D39.

Fig. S3. The fluorescence-based antimicrobial assay for IC_{50} determination is not dependent on the basal level of fluorescence signal generated by untreated bacteria. (A) Representative plots of fluorescence over time for incubations of *Spn* D39 (IU1690, black), TIGR4 (IU11966, red) and TIGR4 Δ*cps* (IU12001, blue) in NPB showing variability in fluorescence signals among strains. (B) Representative plots showing similar fluorescence signals between D39 (IU1690, black) and Δ*amiA-F* (IU11759, red) in NPB. (C) Mean (± SEM) fluorescence (AFU at approximately 4.5 h) in NPB for *Spn* strains and serotypes and other *Streptococcus* species (y-axis) plotted as a function of the mean IC_{50} for CXCL10 (x-axis; closed symbols). Open symbols indicate estimated IC_{50} values. Upper and lower panels show data from different batches of CXCL10. Linear regression analysis showed no correlation (upper panel, R^2=0.14; lower panel R^2=0.06).

Fig. S4. The Δ*amiA-F* clean deletion mutant phenocopies the Δ*amiA-F*::P_c-*aad9* strain, and the Δ*amiA-F* mutation is responsible for the phenotype seen in the ΔOPT mutant. CXCL10 killing of the Δ*amiA-F* strain is consistent with both fluorescence-based and CFU survival assays. (A) D39 *rpsL1* (IU1781, black) and isogenic Δ*amiA-F* clean deletion strain (IU13780, red) were assayed with a fluorescence-based antimicrobial

assay in NPB with various concentrations of CXCL10, LL-37 and nisin. (B) D39 (IU1690, black), Δ*amiA-F* (IU11759, red), Δ[*spd_1166-spd_1170*] (IU11778, green), Δ*aliA* (IU11848, purple), Δ*aliB* (IU11850, light blue) and ΔOPT (IU11919, dark blue) were assayed with a fluorescence-based antimicrobial assay in NPB with 6 μM CXCL10, 1.2 μM LL-37 and 2.4 μM nisin. (C) *Spn* D39 (IU1690, black) and Δ*amiA-F* (IU11759, red) were assayed with a fluorescence-based antimicrobial assay (Fluor.) and a CFU survival assay (CFU) in NPB (see Materials and Methods) with 6 μM CXCL10. Experiments in A, B, and C were performed once with duplicate wells.

Fig. S5. *Spn* D39 ∆*amiA-F* mutants in NPB are more resistant to nisin, whereas *Spn* D39 ∆*dlt* and *Spn* TIGR4 are more sensitive to LL-37 and nisin relative to *Spn* D39. Sensitivity to LL-37 (A and B) and nisin (C and D) in NPB was determined using a fluorescence-based antimicrobial assay, as described in Materials and Methods. (A and C): *S. pneumoniae* D39 (IU1690, black circles; data from Fig. 2), *Spn* D39 Δ*amiA-F* (IU11759, red squares), *Spn* D39 ΔOPT (IU11919, blue triangles), and *Spn* D39 Δ*dltA* (IU12470, green inverted triangles). (B and D): D39 (IU1690, black circles; data from Fig. 2), *Spn* TIGR4 (IU11966, red squares), and *Spn* TIGR4 Δ*cps* (IU12001, blue triangles). n indicates the number of independent experiments, each containing duplicate reactions. Each data point represents the mean ± SEM (if not visible, error bars are smaller than the symbol). Dose-response curves were fit to pooled data in GraphPad Prism, using the (log of inhibitor vs. response-variable slope) function. See Table 3 for 95% CI values.

Fig. S6. Sensitivity to CXCL10 and dose response curves of *S. mitis*, *S. sanguinis*, and *S. mutans* to LL-37. Sensitivities of *S. mitis* (IU11303, red), *S. sanguinis* (IU11305,

blue), and *S. mutans* (IU11309, green) to (A) CXCL10 and (B) LL-37 in NPB buffer relative to *Spn* D39 (IU1690, black closed circles; data from Fig. 2B) and *Spn* TIGR4 (IU11966, black open circles; data from Fig. S5B) were determined using a fluorescence-based antimicrobial assay, as described in Materials and Methods. Each bar or point represents the mean ± SEM of n independent experiments, each containing duplicate reactions. Dose-response curves and IC_{50} values in (B) were fit to a dose-response curve (log of inhibitor vs. response-variable slope) using GraphPad Prism. IC_{50} of *S. mutans* to LL-37 was estimated to be 5 µM.

Fig. S7. Δ*ftsX* and Δ*ftsE* mutants of *B. subtilis* show similar IC_{50} values to CXCL10 or N-CXCL10 as the *ftsE*$^+$ *ftsX*$^+$ parent. CFU survival assays were performed as described in Materials and Methods. (A) *ftsX*$^+$ parent (IU12153; black circles) and Δ*ftsX*::Tn10 *spec* (IU12165; red squares) mutant of *B. subtilis* in NPB buffer. Data shown was obtained from one experiment with duplicate wells. (B) and (C), assays in TGS buffer, parent (IU12153; black), Δ*ftsX* (IU12981; red) and *ftsE*::Tn10 *spec* (IU12166; blue). n indicates the number of independent experiments. IC_{50} and 95% CI values in comparison with other assay conditions are included in Table S3. (D) and (E) 3 x 10^4 CFU per reaction of *Bsu* parent and Δ*ftsX* strains in DMEM +10% (vol/vol) FBS with indicated CXCL10 concentrations were incubated for 3.4 h statically in 96-well plates at 37ºC in 5% CO_2 before the addition of resazurin dye. Fluorescence was measured (shown in D) and cells were serially diluted and plated for CFU survival assay (shown in E) at 5.2 h. Results included in Table S3.

Fig. S8. CXCL10 does not show antimicrobial activity against *Spn* D39 in DMEM ± 10% (vol/vol) FBS. (A) *Spn* D39 (IU1690) was incubated in DMEM +10% (vol/vol) FBS

with 11.6 µM CXCL10 (three independent experiments with duplicate wells) or various concentrations of nisin (one experiment with duplicate wells) and assayed with a fluorescence-based antimicrobial assay as described in Materials and Methods. Statistical significance was determined using Mann-Whitney t-test of CXCL10-treated vs untreated sample. **, $p < 0.01$. (B) *Spn* D39 (IU1690) was incubated in DMEM with no FBS with 3.5 µM CXCL10 or 3 µM nisin for 3.8 h. Resazurin dye was then added, fluorescence was measured 2 h later (top panel), and cells were serially diluted and plated for CFU (bottom panel). Numbers on bars indicate the percentage of fluorescence relative to the untreated sample (top panel) or 10^7 CFU/mL (bottom panel). This experiment was performed once with duplicate wells.

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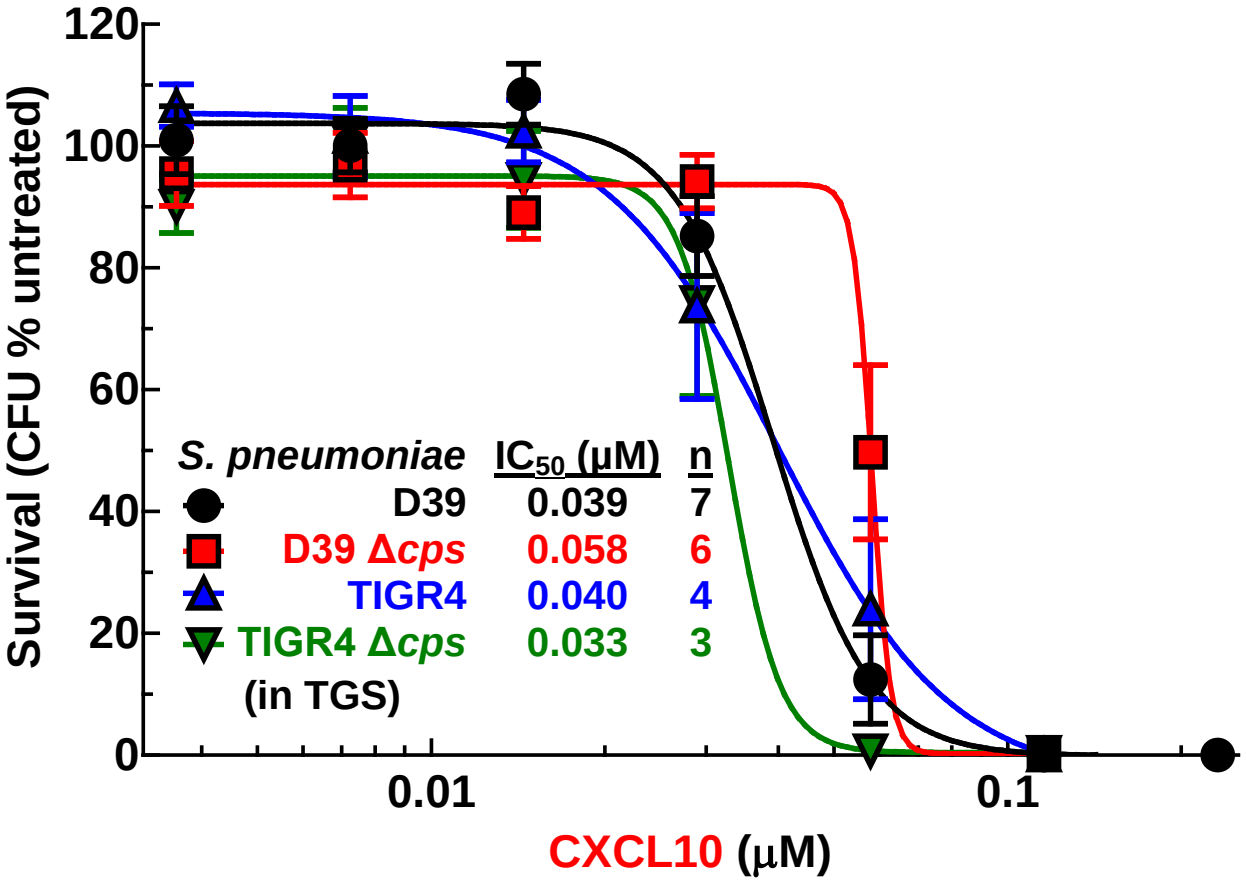
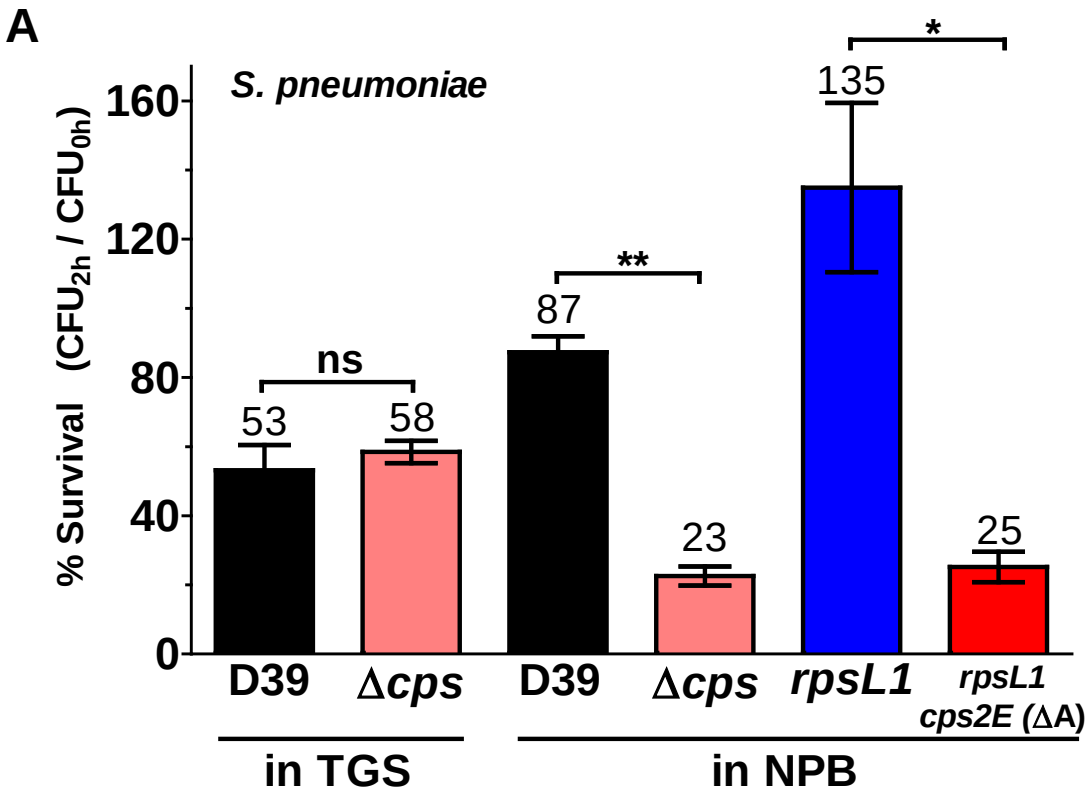

120
100
80
60
40
20
0
Survival (CFU % untreated)
0.01
0.1
CXCL10 (μM)
S. pneumoniae
IC50 (μM)
n
D39
0.039
7
D39 Δcps
0.058
6
TIGR4
0.040
4
TIGR4 Δcps
0.033
3
(in TGS)

Fig. S1


A
S. pneumoniae
% Survival (CFU2h / CFU0h)
160
120
80
40
0
ns
**
*
53
58
87
23
135
25
D39
ΔcpsD39
Δcps
rpsL1
rpsL1 cps2E (ΔA)
in TGS
in NPB

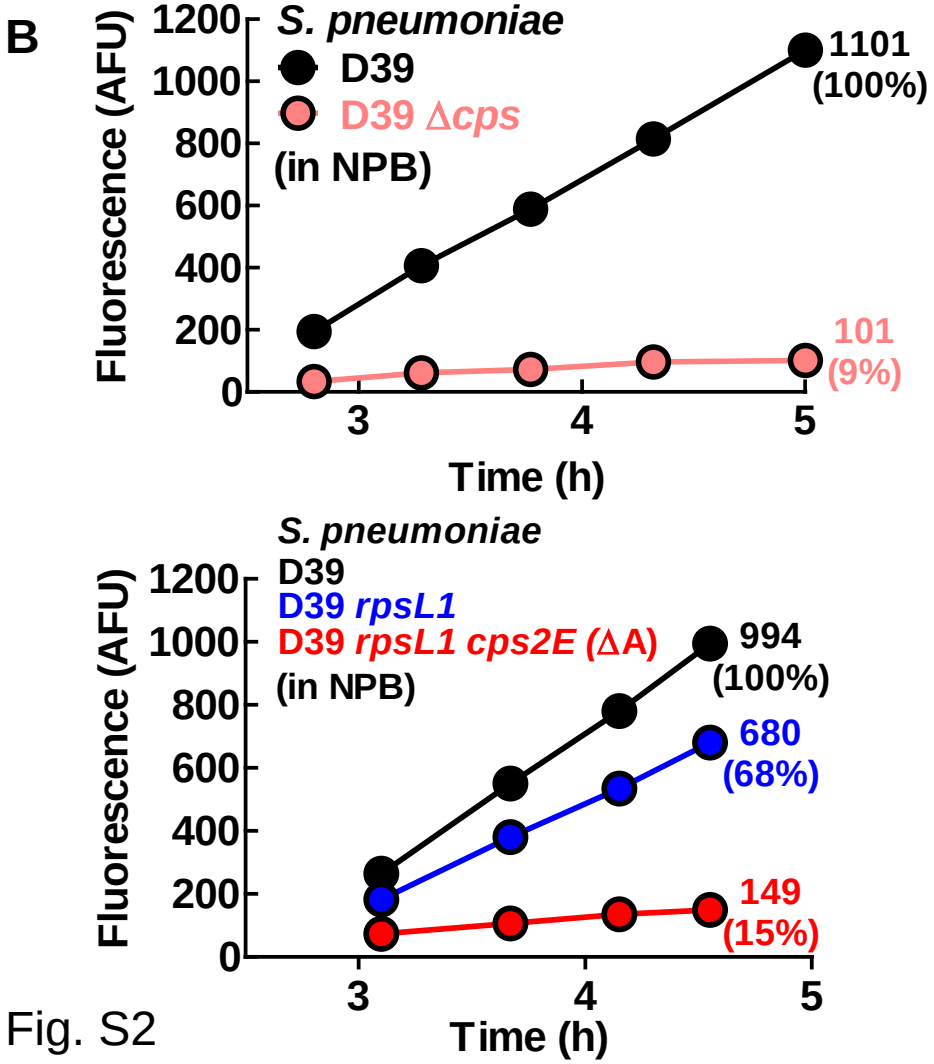

B
S. pneumoniae
D39
D39 Δcps
(in NPB)
Fluorescence (AFU)
1200
1000
800
600
400
200
0
1101
(100%)
101
(9%)
3
4
5
Time (h)
S. pneumoniae
D39
D39 rpsL1
D39 rpsL1 cps2E (ΔA)
(in NPB)
994
(100%)
680
(68%)
149
(15%)

Fig. S2

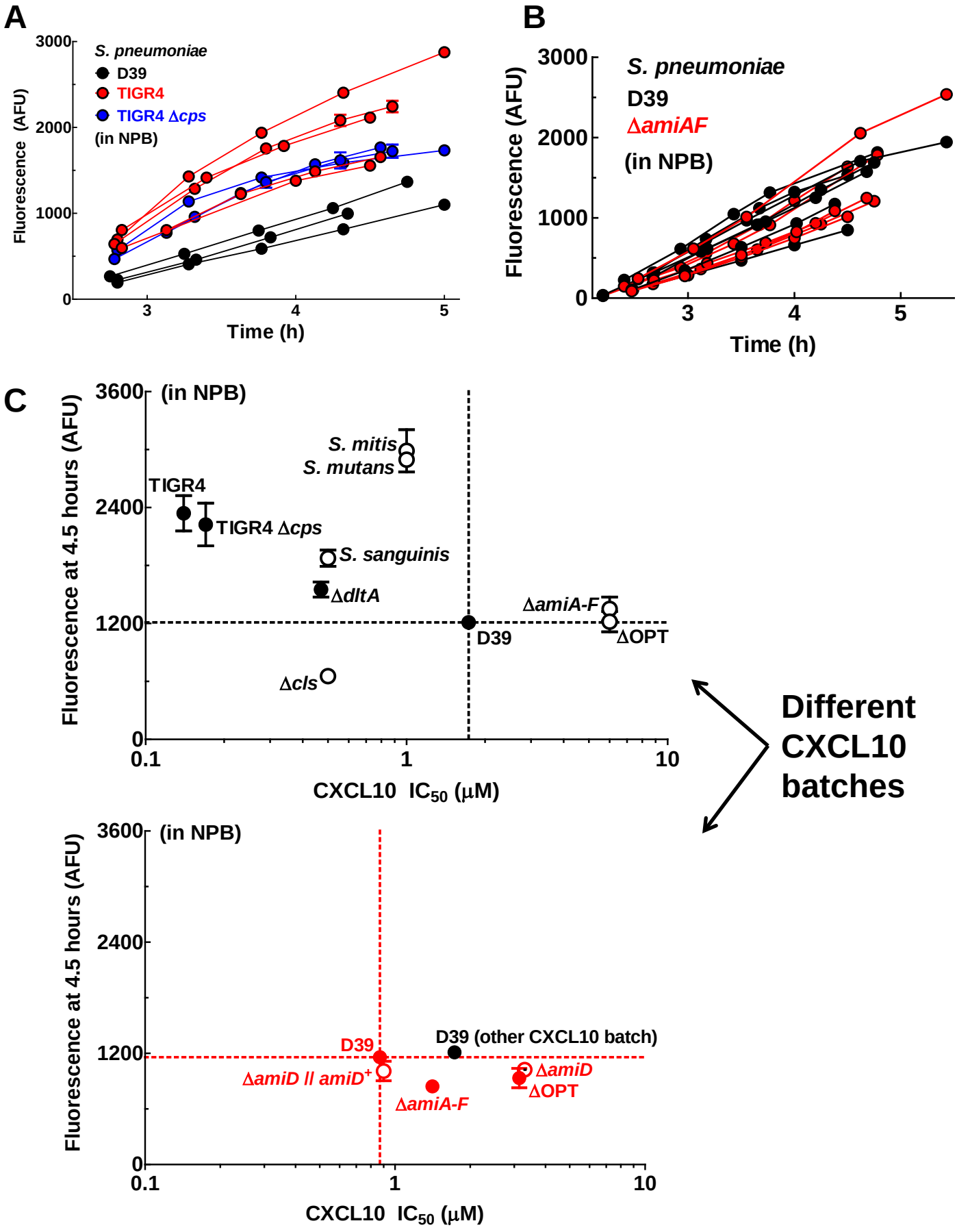
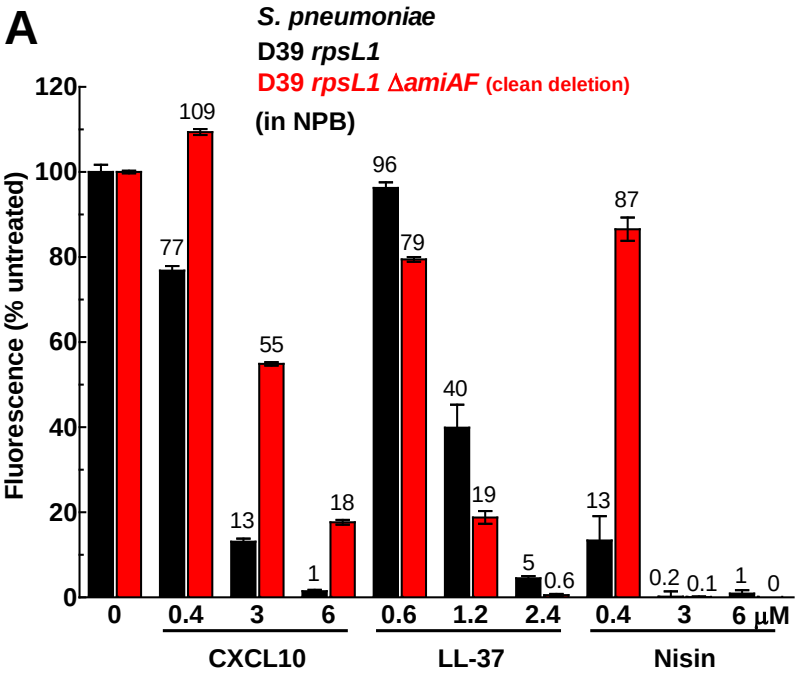
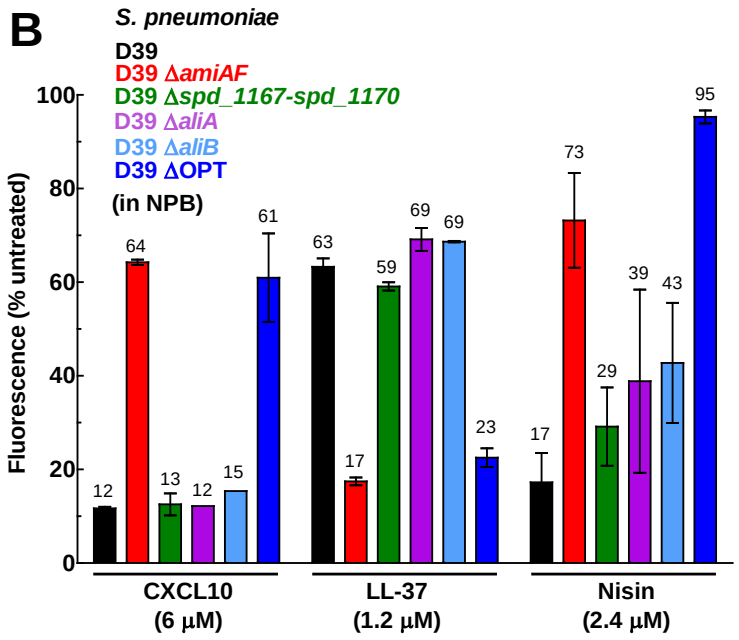

A
S. pneumoniae
D39
TIGR4
TIGR4 Δcps
(in NPB)
Fluorescence (AFU)
Time (h)
B
S. pneumoniae
D39
ΔamiAF
(in NPB)
Fluorescence (AFU)
Time (h)
C
(in NPB)
Fluorescence at 4.5 hours (AFU)
S. mitis
S. mutans
TIGR4
TIGR4 Δcps
S. sanguinis
ΔdltA
ΔamiA-F
ΔOPT
D39
Δcls
CXCL10 IC50 (μM)
Different CXCL10 batches
(in NPB)
Fluorescence at 4.5 hours (AFU)
D39
D39 (other CXCL10 batch)
ΔamiD II amiD+
ΔamiA-F
ΔamiD
ΔOPT
CXCL10 IC50 (μM)

Fig. S3


A
S. pneumoniae
D39 rpsL1
D39 rpsL1 ΔamiAF (clean deletion)
(in NPB)
Fluorescence (% untreated)
120
100
80
60
40
20
0
109
77
55
13
18
1
96
79
40
19
5
0.6
87
13
0.2
0.1
1
0
0
0.4
3
6
0.6
1.2
2.4
0.4
3
6 μM
CXCL10
LL-37
Nisin


B
S. pneumoniae
D39
D39 ΔamiAF
D39 Δspd_1167-spd_1170
D39 ΔaliA
D39 ΔaliB
D39 ΔOPT
(in NPB)
Fluorescence (% untreated)
100
80
60
40
20
0
12
64
13
12
15
61
63
17
59
69
69
23
17
73
29
39
43
95
CXCL10
(6 μM)
LL-37
(1.2 μM)
Nisin
(2.4 μM)

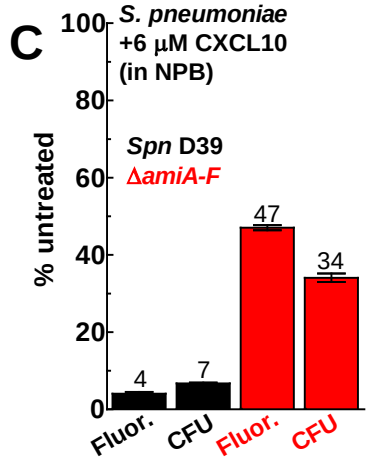

C
S. pneumoniae
+6 μM CXCL10
(in NPB)
Spn D39
ΔamiA-F
% untreated
100
80
60
40
20
0
4
7
47
34
Fluor.
CFU
Fluor.
CFU

Fig. S4

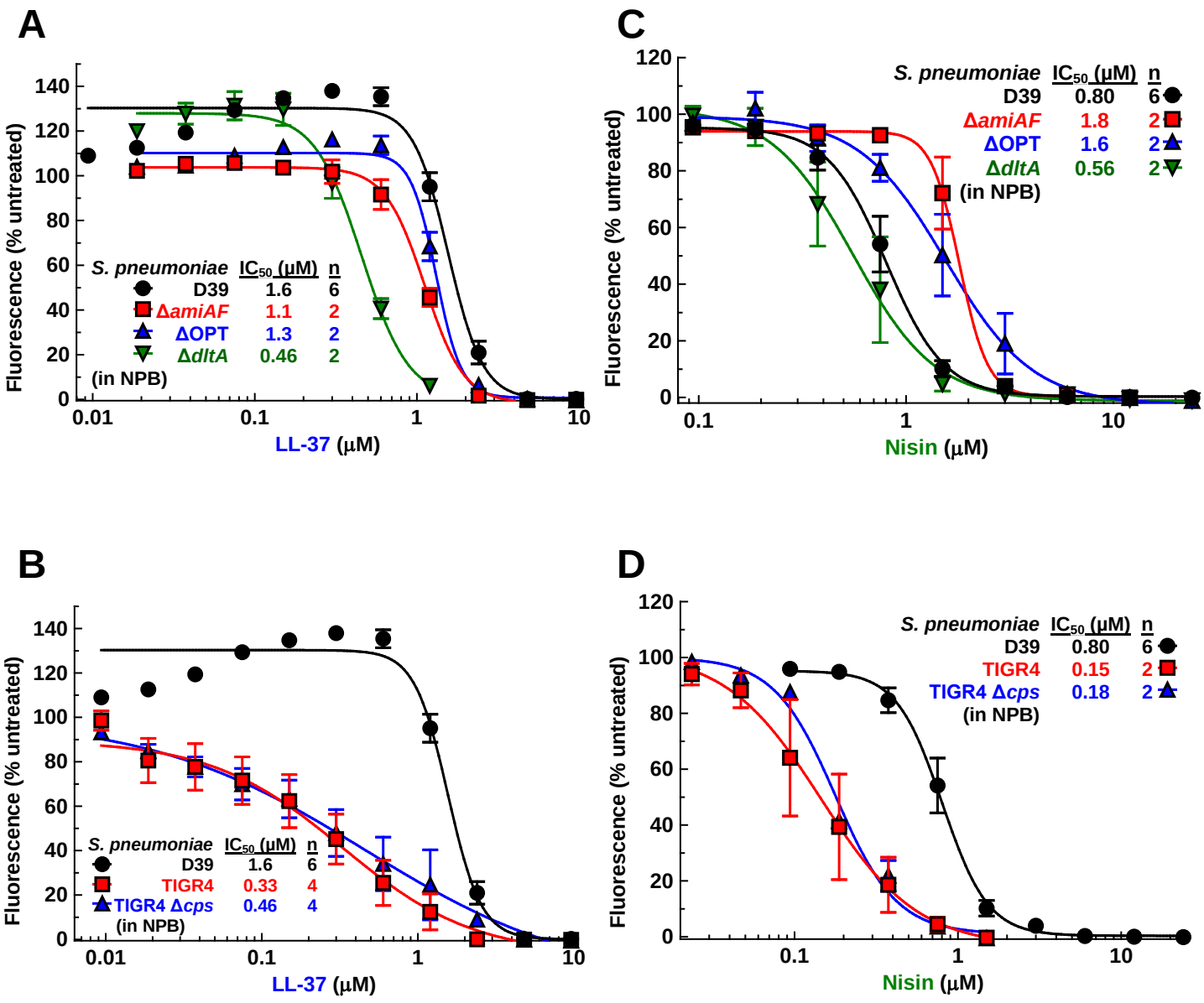

A
Fluorescence (% untreated)
LL-37 (μM)
S. pneumoniae IC50 (μM) n
D39 1.6 6
ΔamiAF 1.1 2
ΔOPT 1.3 2
ΔdltA 0.46 2
(in NPB)
C
Nisin (μM)
S. pneumoniae IC50 (μM) n
D39 0.80 6
ΔamiAF 1.8 2
ΔOPT 1.6 2
ΔdltA 0.56 2
(in NPB)
B
LL-37 (μM)
S. pneumoniae IC50 (μM) n
D39 1.6 6
TIGR4 0.33 4
TIGR4 Δcps 0.46 4
(in NPB)
D
Nisin (μM)
S. pneumoniae IC50 (μM) n
D39 0.80 6
TIGR4 0.15 2
TIGR4 Δcps 0.18 2
(in NPB)

Fig. S5

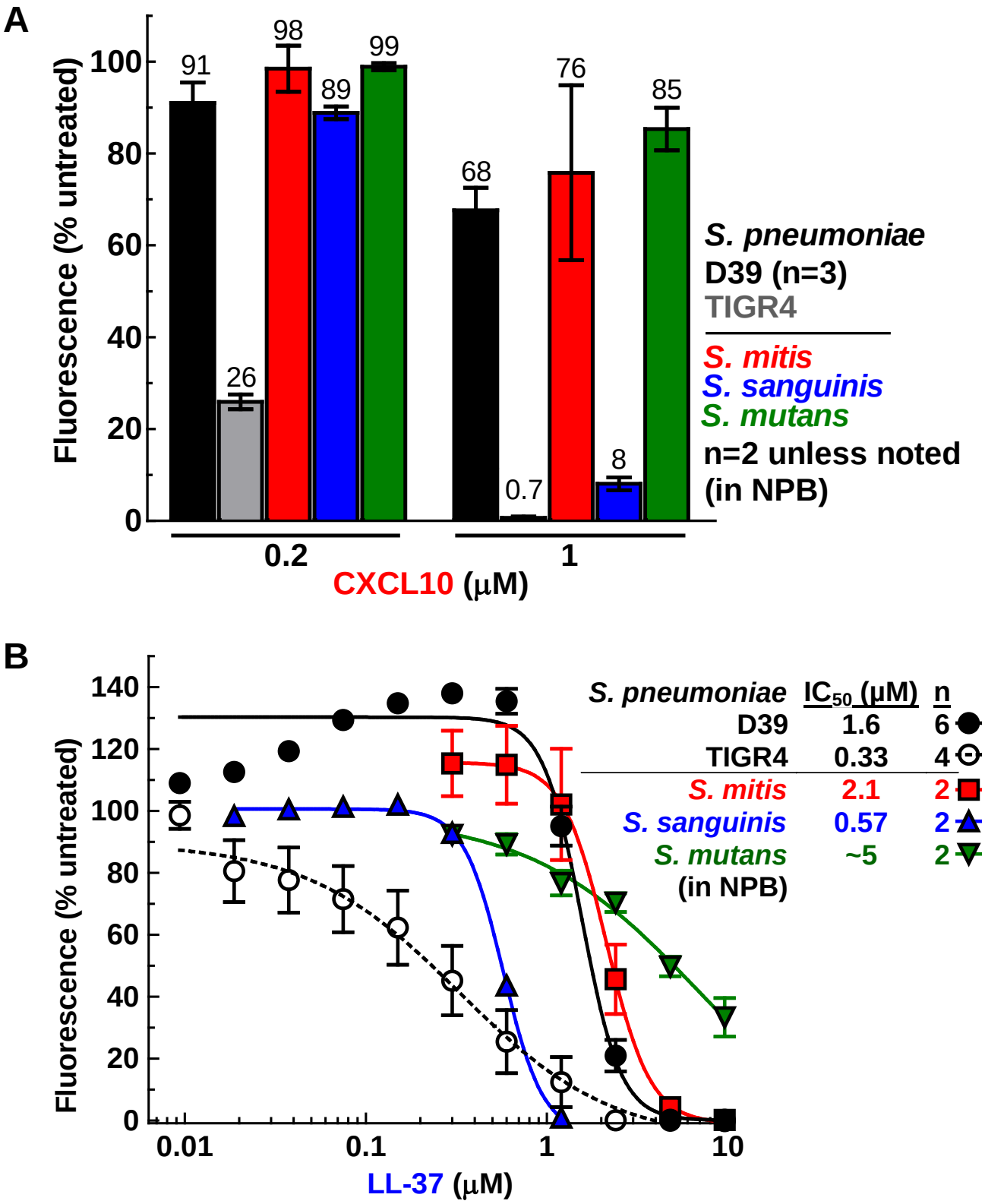
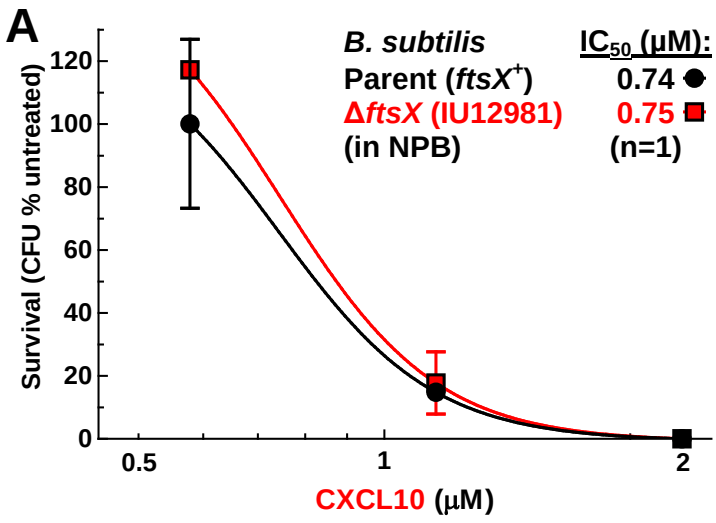
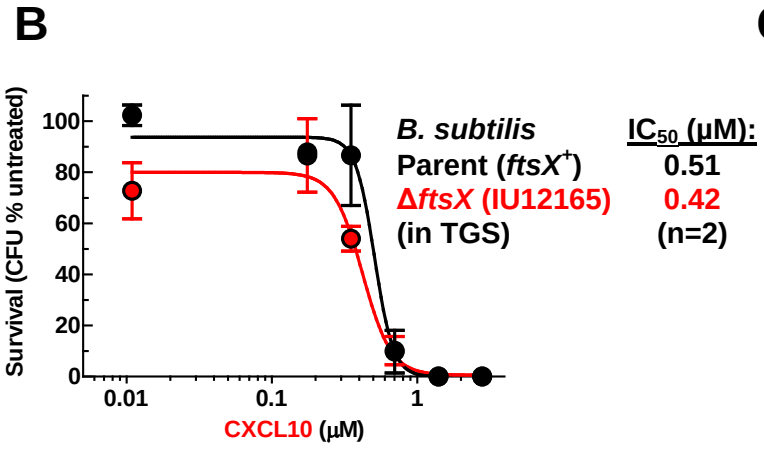
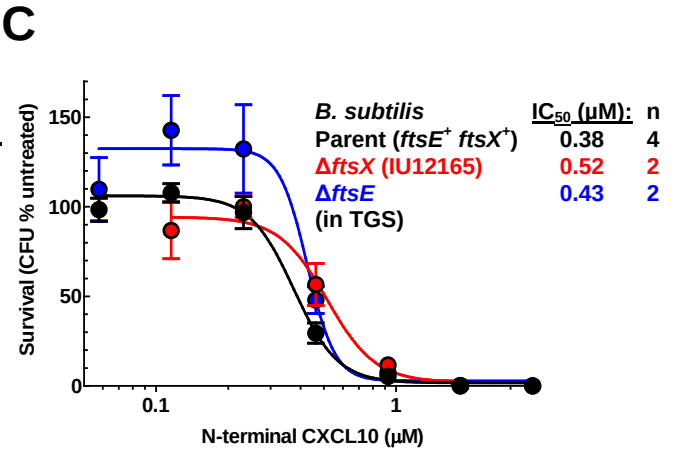
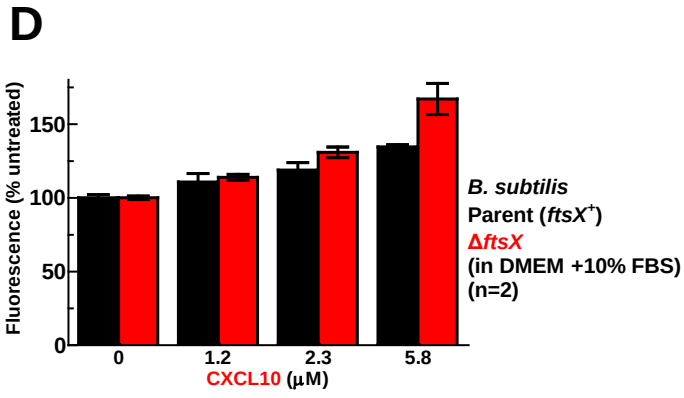

A
Fluorescence (% untreated)
100
80
60
40
20
0
91
26
98
89
99
68
0.7
76
8
85
0.2
1
CXCL10 (μM)
S. pneumoniae
D39 (n=3)
TIGR4
S. mitis
S. sanguinis
S. mutans
n=2 unless noted
(in NPB)
B
Fluorescence (% untreated)
140
120
100
80
60
40
20
0
0.01
0.1
1
10
LL-37 (μM)
S. pneumoniae IC50 (μM) n
D39 1.6 6
TIGR4 0.33 4
S. mitis 2.1 2
S. sanguinis 0.57 2
S. mutans ~5 2
(in NPB)

Fig. S6


A
B. subtilis
Parent (ftsX+)
ΔftsX (IU12981)
(in NPB)
IC50 (μM):
0.74
0.75
(n=1)
Survival (CFU % untreated)
CXCL10 (μM)


B
B. subtilis
Parent (ftsX+)
ΔftsX (IU12165)
(in TGS)
IC50 (μM):
0.51
0.42
(n=2)
Survival (CFU % untreated)
CXCL10 (μM)


C
B. subtilis
Parent (ftsE+ ftsX+)
ΔftsX (IU12165)
ΔftsE
(in TGS)
IC50 (μM):
n
0.38
4
0.52
2
0.43
2
Survival (CFU % untreated)
N-terminal CXCL10 (μM)


D
Fluorescence (% untreated)
B. subtilis
Parent (ftsX+)
ΔftsX
(in DMEM +10% FBS)
(n=2)
CXCL10 (μM)

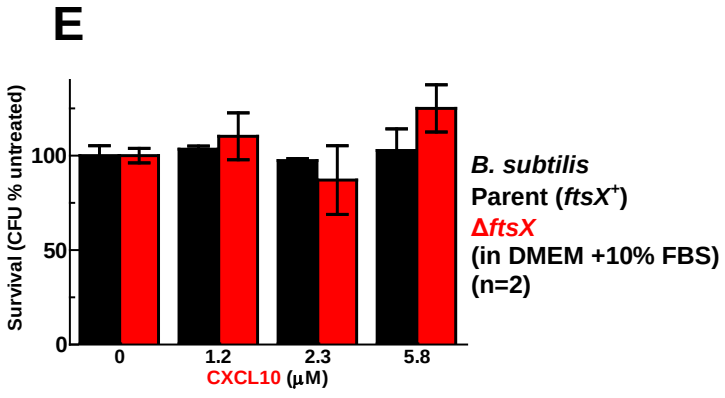
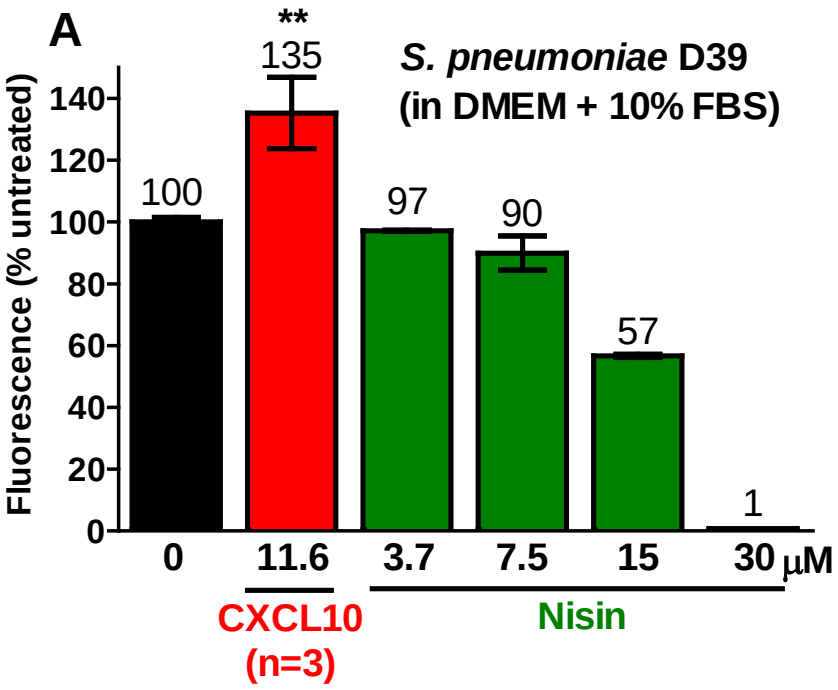
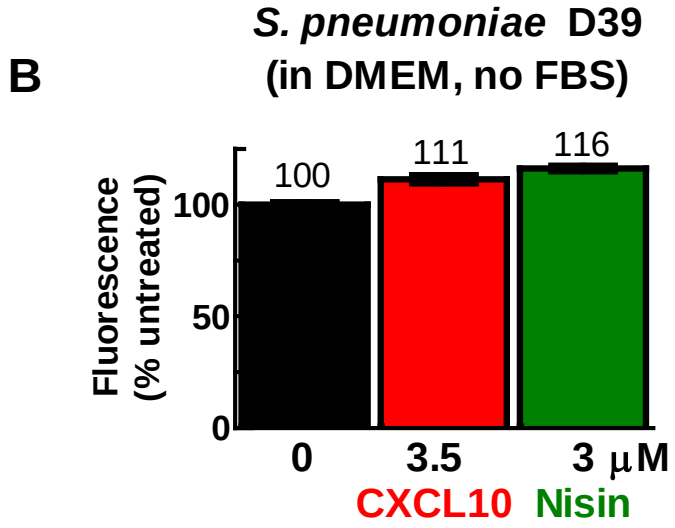

E
Survival (CFU % untreated)
B. subtilis
Parent (ftsX+)
ΔftsX
(in DMEM +10% FBS)
(n=2)
CXCL10 (μM)

Fig. S7


A
**
135
S. pneumoniae D39
(in DMEM + 10% FBS)
Fluorescence (% untreated)
140
120
100
80
60
40
20
0
100
97
90
57
1
0
11.6
3.7
7.5
15
30 μM
CXCL10
(n=3)
Nisin


S. pneumoniae D39
(in DMEM, no FBS)
B
Fluorescence
(% untreated)
100
50
0
100
111
116
0
3.5
3 μM
CXCL10
Nisin

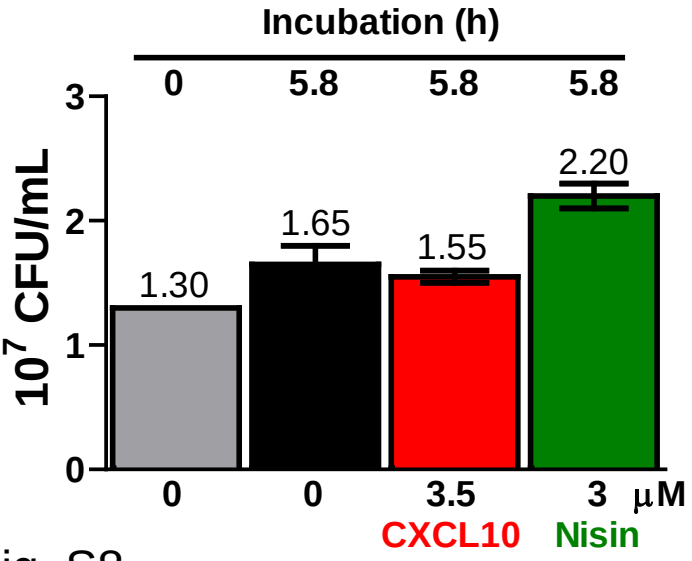

Incubation (h)
0
5.8
5.8
5.8
10^7 CFU/mL
3
2
1
0
1.30
1.65
1.55
2.20
0
0
3.5
3 μM
CXCL10
Nisin

Fig. S8