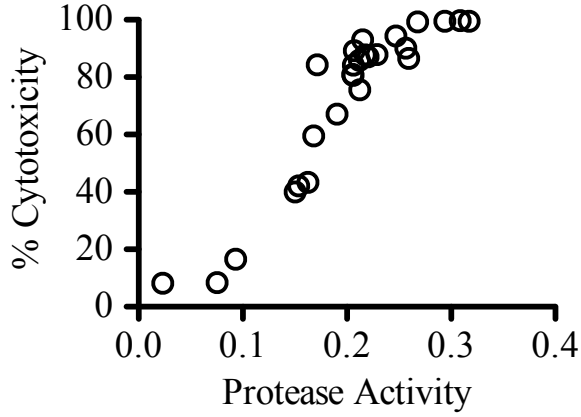

Figure S1. Correlation between protease production and cytotoxicity by ocular clinical *S. marcescens* isolates and strain PIC3611.
Significant correlatoin of cytotoxicity to HCLE cells to secreted protease activity (Pearson r=0.925, p<0.001).

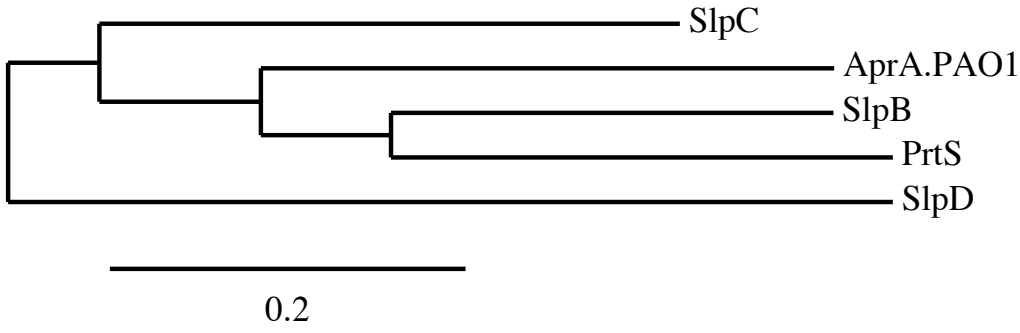

Figure S2. Phylogram of putative *S. marcescens* metalloproteases. Comparison of amino acid sequences of alkaline protease (AprA) from *Pseudomonas aeruginosa*, serralysin (PrtS) and candidate metalloproteases from *S. marcescens* using the "one-click" mode tree rendering program from www.Phylogeny.fr using default settings. Number of substitutions per site are proportional to the branch length.

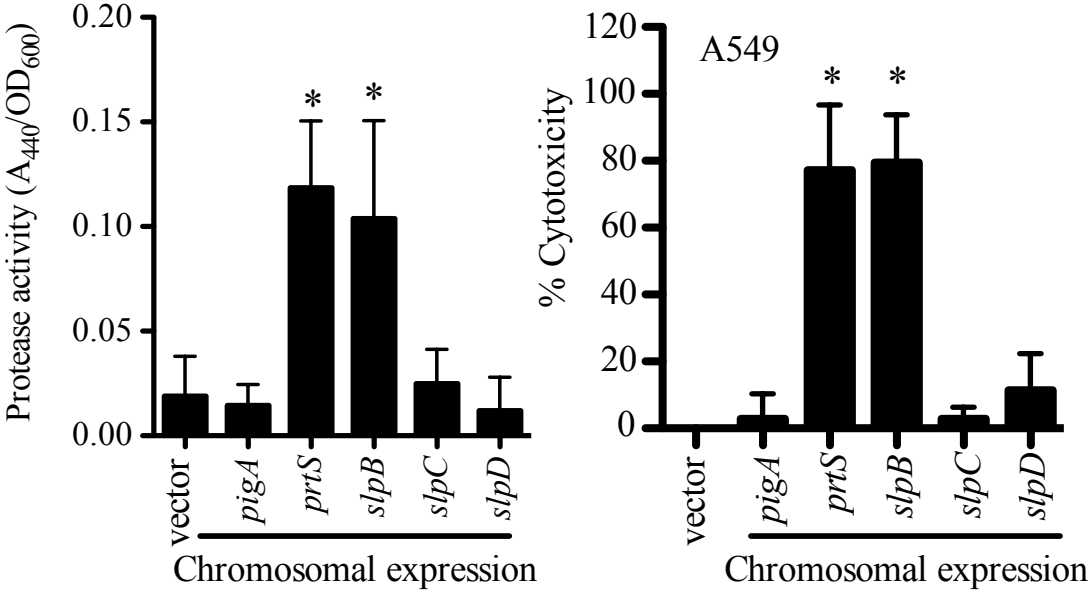

Figure S3. Cytotoxicity to human epithelial cell lines and protease activity of induced putative metalloprotease genes. Induction of putative metalloprotease genes (no His_7-tag) from the PIC3611 chromosome was followed by measuring cytotoxicity of epithelial cells challenged by filtered and normalized supernatants of cultures grown overnight. **A.** Protease activity measured using azocasein. **B**. Cytotoxicity to A549 airway cells were challenged with secretomes. Negative controls include the episome pMQ131 (vector) that, like pMQ200, has a kanamycin resistance gene as a selectable marker, and chromosomal induction of the non-protease gene *pigA*. For each panel the average and standard deviation are shown (n≥3 independent experiments). Asterisk indicates that the supernatants were significantly more cytotoxic than the vector control supernatant as determined by ANOVA with Tukey's post-test.

Figure S4

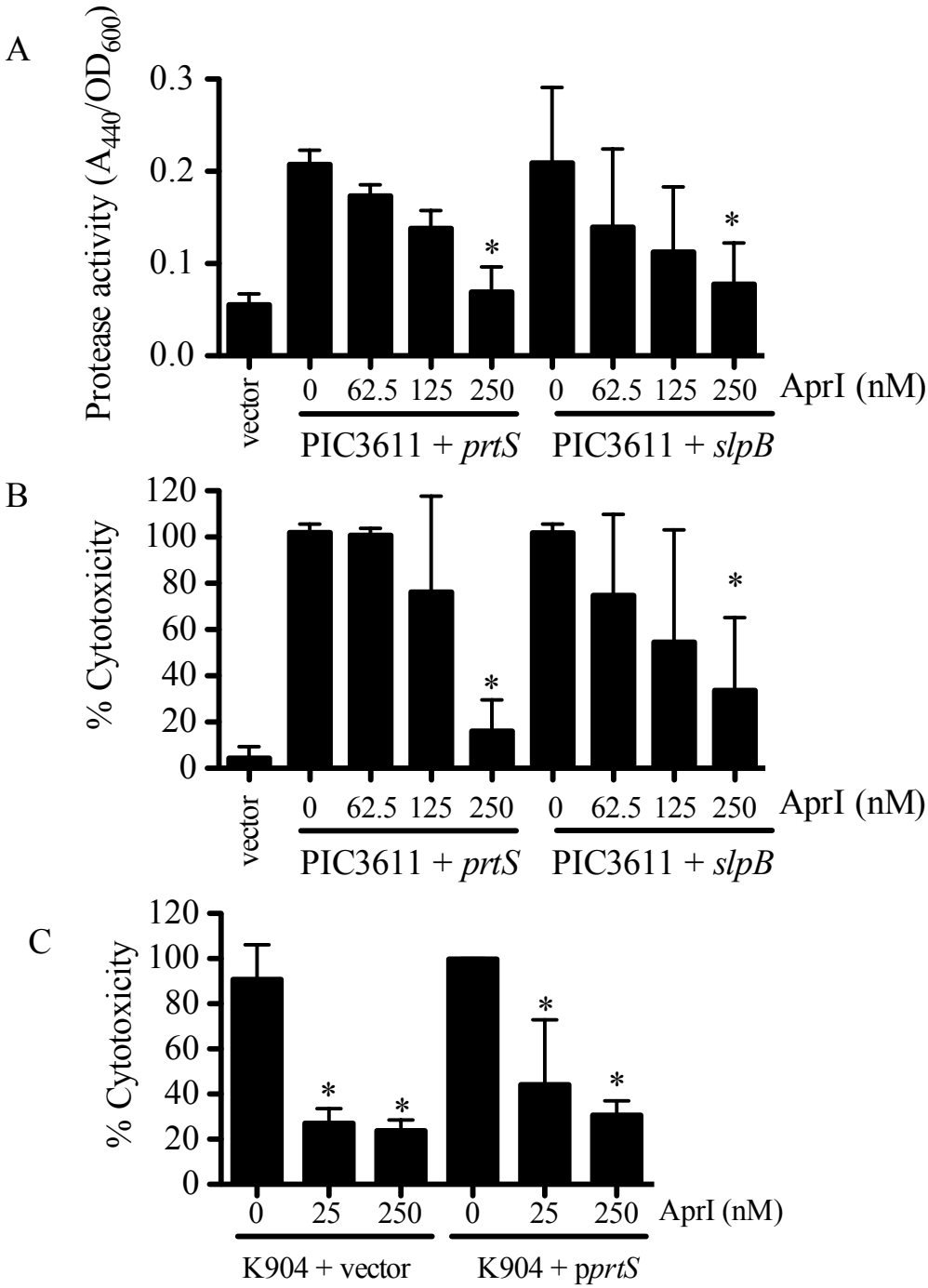

Figure S4. AprI inhibition of PrtS and SlpB activity and cytotoxicity by K904 supernatants. A. Protease activity as measured using azocasein. AprI inhibition of protease activity of filtered and normalized supernatants from PIC3611 with pMQ125 (vector), pMQ356 (p*prtS*), and pMQ436 (p*slpB*), n=3 for vector, 5 for other groups. **B.** Inhibition of cytotoxicity to A549 cells from PrtS and SlpB induced from PIC3611, n=8. **C.** Cytotoxicity to A549 cells by secretomes from K904 with either pMQ125 (vector) or pMQ356 (p*prtS*). Means and averages are shown; asterisk indicates significantly different from the respective 0 nM AprI addition group by ANOVA with Tukey's post-test ($p<0.05$).

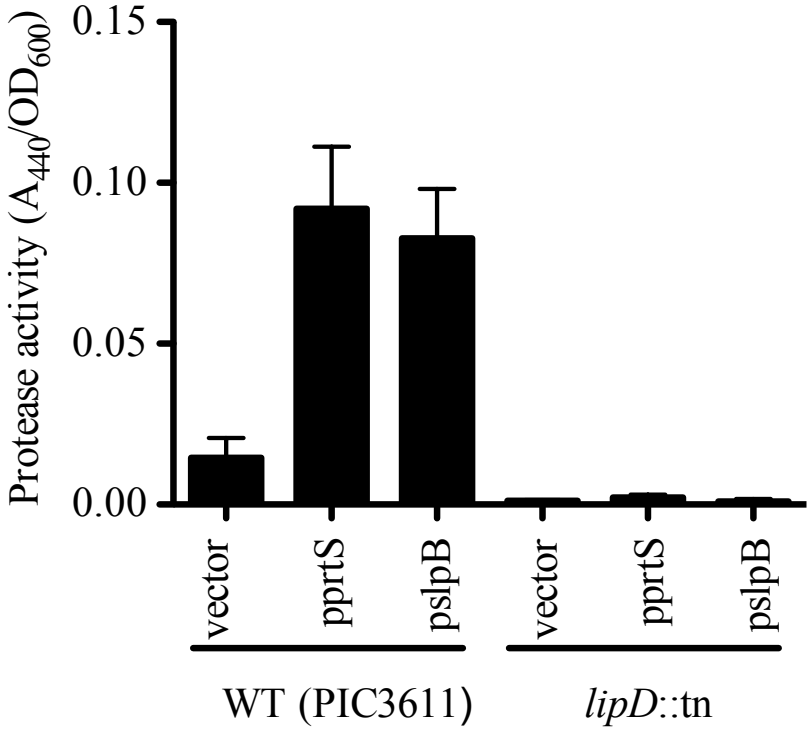

Figure S5. LipD is necessary for secretion of SlpB. Protease activity from bacterial supernatants using azocasein as a substrate normalized to culture densities. N=9 independent replicates from 3 separate experiments. Mean and standard deviations are shown. Vector = pMQ125, p*prtS* = pMQ356, p*slpB* = pMQ436.

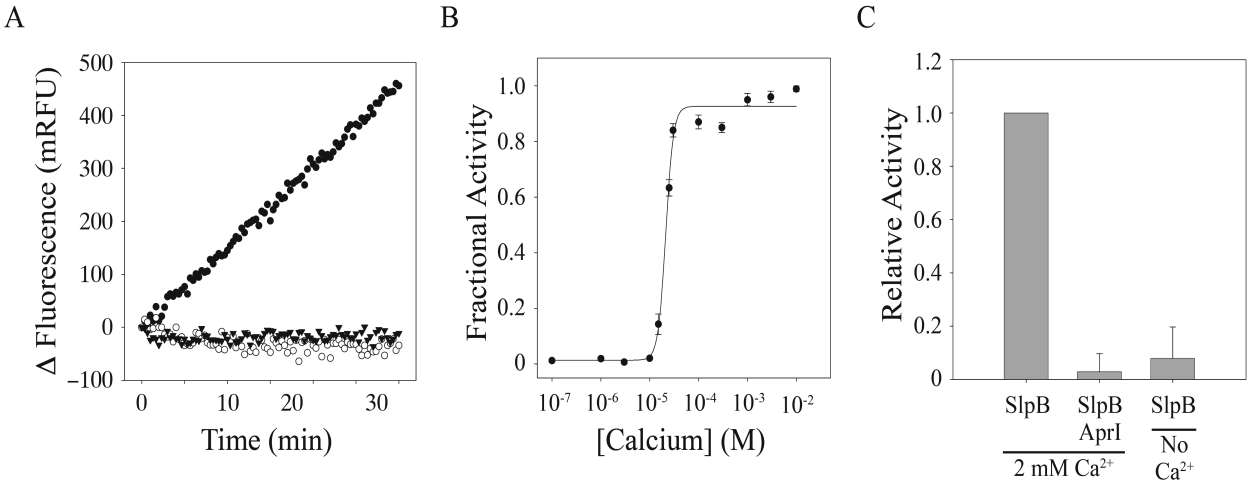

Figure S6. SlpB is a Ca^{2+}-regulated protease. The Ca^{2+}-induced activation of SlpB was assessed in vitro. **A.** Protease activity was assessed after refolding in the presence and absence of 2 mM Ca^{2+} using a fluorescent peptide substrate. Protease refolded in the presence of 2 mM Ca^{2+} (closed circles) resulted in active protease, as measured kinetically as an increase in substrate fluorescence. This activity was not seen when the protease was refolded in the absence of Ca^{2+} (open circles) or in buffer controls (closed triangles). **B.** Calcium titrations were performed to assess the apparent affinity of SlpB and its activation by Ca^{2+}. The activation of SlpB by Ca^{2+} was fit with the Hill equation and determined the apparent Ca^{2+} affinity to be 22 μM with a Hill coefficient of 5.5. **C.** Protease activity was assessed in the presence and absence of the *P. aeruginosa* AprI protease inhibitor. Inclusion of stoichiometric concentrations of the inhibitor resulted in complete inhibition of the SlpB protease. Data shown are mean +/- standard deviation from at least three independent experiments.

Table S1. Oligonucleotide primers used in this study.

Primer Number	Primer Sequence[a]
2638[b]	AACTGGAGGAAGGTGGGGAT
2639[b]	AGGAGGTGATCCAACCGCA
3174	GGCCTGTTCGACTACAGCTC
3175	TAGCGTAGGTGATGCTGACG
3186	GCTTTCATTGCAATCCTGGT
3187	TAATTGCCGGGATGAGAAAG
3177	GATGCCGCCTATTATCAGGA
3178	CTGTAGCCGGAGAAGTCCAG
1791	GACGACCTGCTGCATTATCA
1793	GGCTGGCCAAATACCTTGTA
1908	aaattctgttttatcagaccgcttctgcgttctgatTTACACGATAAAGTCAGTGGCGAC
1909	ttgtgagcggataacaatttcacacaggaaacagctATGTCTATCTGTCTGATTGATATC
2352	ctctctactgtttctccatacccgtaggaggaaaaaATGTCTATCTGTCTGATTGATATC
2500	tttctccatacccgtaggaggaaaaaatgaccatgCACCACCATCACCACCATCACGCCGCCACAACCGGTTACG
1542	agtgccaagcttgcatgcctgcaggtcgactctagaCGATCGCAGTTGGCACGACAAGG
1543	acacaggaaacagctatgaccatgattacgaattcgaGCACCGTAGAAATGCTGGATCG
1912	ttctgttttatcagaccgcttctgcgttctgatTTACGCCAACACGAAATCGCCGGCATC
1913	attgtgagcggataacaatttcacacaggaaacagctGTTATGTCATCAAGCAATGCTTC
2355	caactctctactgtttctccatacccgtaggaggaaaaaATGTCATCAAGCAATGCTTCC
2493	gaattgtgagcggataacaatttcacacaggaaacagctCGGCAATCGTCACCTGTTGTC
2496	cgcgccggtggcggtgaggaagtcgcgatcgccgcgcaccagctccaggccggcgttttc
3194	gaaaaaatgaccatgcaccaccatcaccaccatcacTCATCAAGCAATGCTTCCGTTAAG
1916	aaattctgttttatcagaccgcttctgcgttctgatCTAGACGAGAATATCGGCGGAAGC
1917	gaattgtgagcggataacaatttcacacaggaaacagctATGTCATTAGCGACCGATAAG
2358	caactctctactgtttctccatacccgtaggaggaaaaaATGTCATTAGCGACCGATAAG

3196	gaaaaaatgaccatgcaccaccatcaccaccatcacTCATTAGCGACCGATAAGATTTCC
1920	aaattctgttttatcagaccgcttctgcgttctgatTCAGGCAAACAAAATATCGCTCTG
1921	gaattgtgagcggataacaatttcacacaggaaacagctATGCGCAGTCGCCACAACGAC
2361	caactctctactgtttctccatacccgtaggaggaaaaaATGCGCAGTCGCCACAACGAC
3198	gaaaaaatgaccatgcaccaccatcaccaccatcacCGCAGTCGCCACAACGACATTTTG
2977	ctgcgcaactgttgggaagggcgatcggtgcgggcctTCATAGTGAATATGTTGTTATCG
2978	ttgtgagcggataacaatttcacacaggaaacagctATGTCTACGCATATTGGCGAGCC

a. lower case letters are regions for yeast recombination and upper case letters are for amplification, underlined letters indicate the polyhistidine tag.

b. from Lin, et. al. (2)

Table S2. Metalloprotease homologs of *S. marcescens* strain Db11.

Gene (ORF #)[a]	Peptidase_M10_C superfamily domain Pfam08548[b]	% AA identity to PrtS / AprA[c]	Predicted Mass of proprotein	HEXXHXUGUXH Zinc-binding motif Sequence[d]
***prtS* (serralysin) SMA4311**	2e-85	100 /54	**54**	HEIGHALGLSH
***slpB* SMA1367**	2e-88	52.6 / 55	**50.4**	HEIGHALGLQH
***slpC* SMA2486**	4e-74	50.3 / 50	**49**	HEIGHALGLSH
***slpD* SMA1606**	2e-63	45 / 44	**57**	HEIGHALGLSH

[a.] ORF number according to the DB11 genome sequence.

[b.] Serralysin C-terminal calcium ion binding domain. Value determined by NCBI BLAST (3).

[c.] Identity determined by BLAST with ≥95% query coverage for each protein. AprA is alkaline protease from *P. aeruginosa*, PrtS is serralysin.

[d.] HEXXHXUGUXH = consensus sequence U= Bulky Hydrophobic Amino Acid, X = Arbitrary Amino Acid.

Supplementary Materials References

1. Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res **36:**W465-469.

2. Lin CS, Horng JT, Yang CH, Tsai YH, Su LH, Wei CF, Chen CC, Hsieh SC, Lu CC, Lai HC. 2010. RssAB-FlhDC-ShlBA as a major pathogenesis pathway in *Serratia marcescens*. Infect Immun **78:**4870-4881.

3. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res **25:**3389-3402.