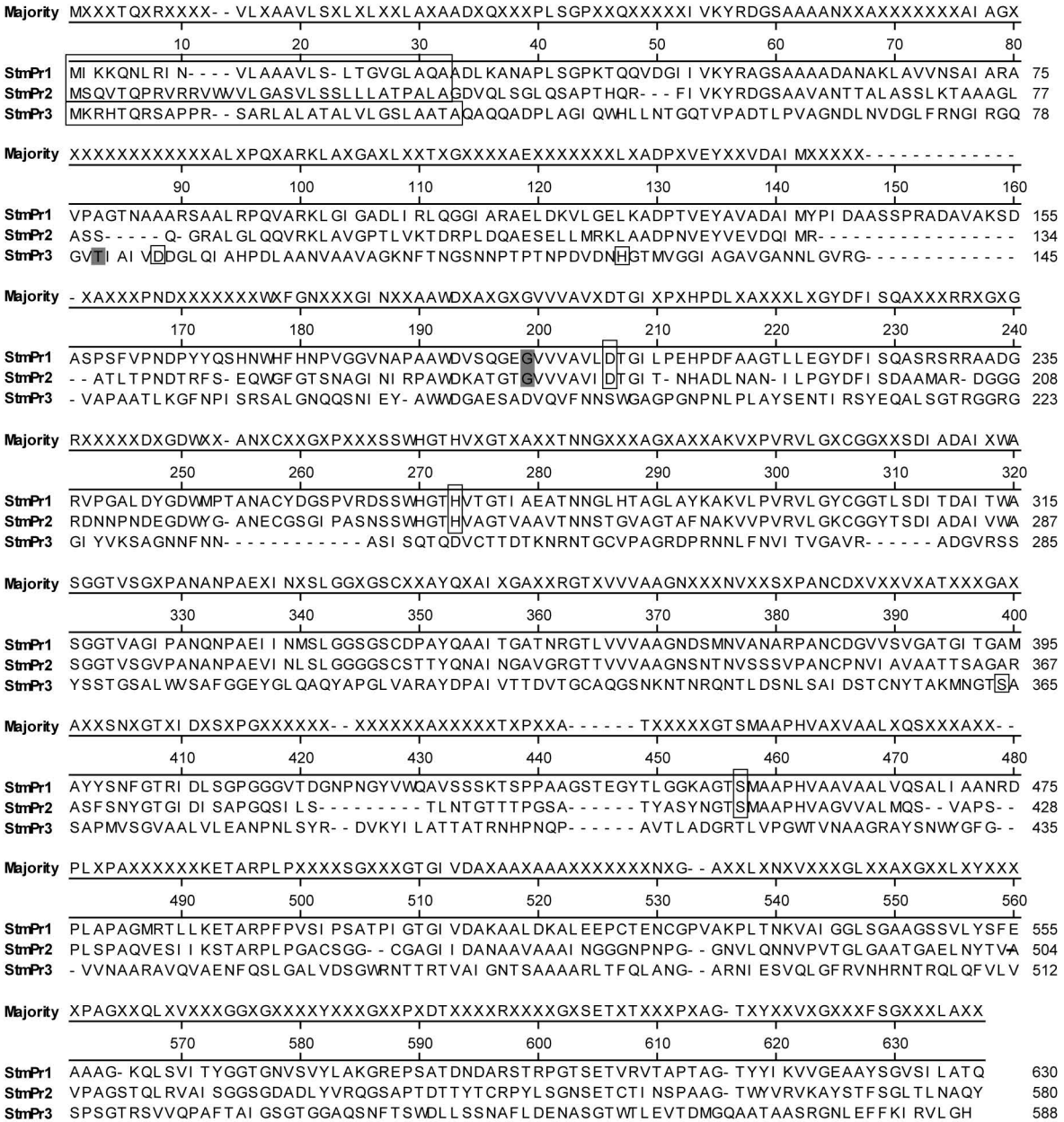

Fig S1. Amino acid sequence alignment of StmPr1, StmPr2, and Stmpr3 from strain K279a. ClustalW alignment of StmPr1, StmPr2, and StmPr3 using DNASTAR Lasergene software. Conserved residues are listed in the majority row, with "X" indicating lack of conservation. Predicted signal sequences and catalytic active site residues are indicated by boxes. Start of the mature proteins is highlighted in grey.

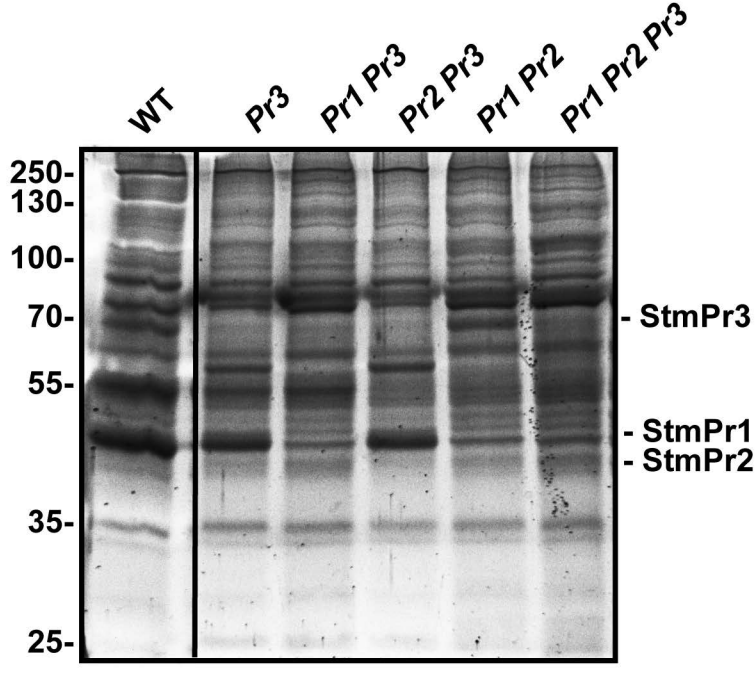

Fig. S2. Secretion profiles of protease mutant strains. Supernatants were collected from WT K279a, stmPr3 mutant NUS11 (Pr3), stmPr1 stmPr3 mutant NUS12 (Pr1 Pr3), stmPr2 stmPr3 mutant NUS13 (Pr2 Pr3), stmPr1 stmPr2 mutant NUS7 (Pr1 Pr2), and stmPr1 stmPr2 stmPr3 mutant NUS14 (Pr1 Pr2 Pr3). Secreted proteins were visualized by SDS-PAGE analysis followed by SYPRO Ruby staining. Bands corresponding to StmPr1, StmPr2, and StmPr3 are indicated. The migration of molecular mass standards (in kDa) is indicated to the left of gel images. Although the samples were examined on the same gel, they were not in adjacent lanes, and therefore we cropped out intervening lanes that were not pertinent to the analysis. Data are representative of three independent experiments.

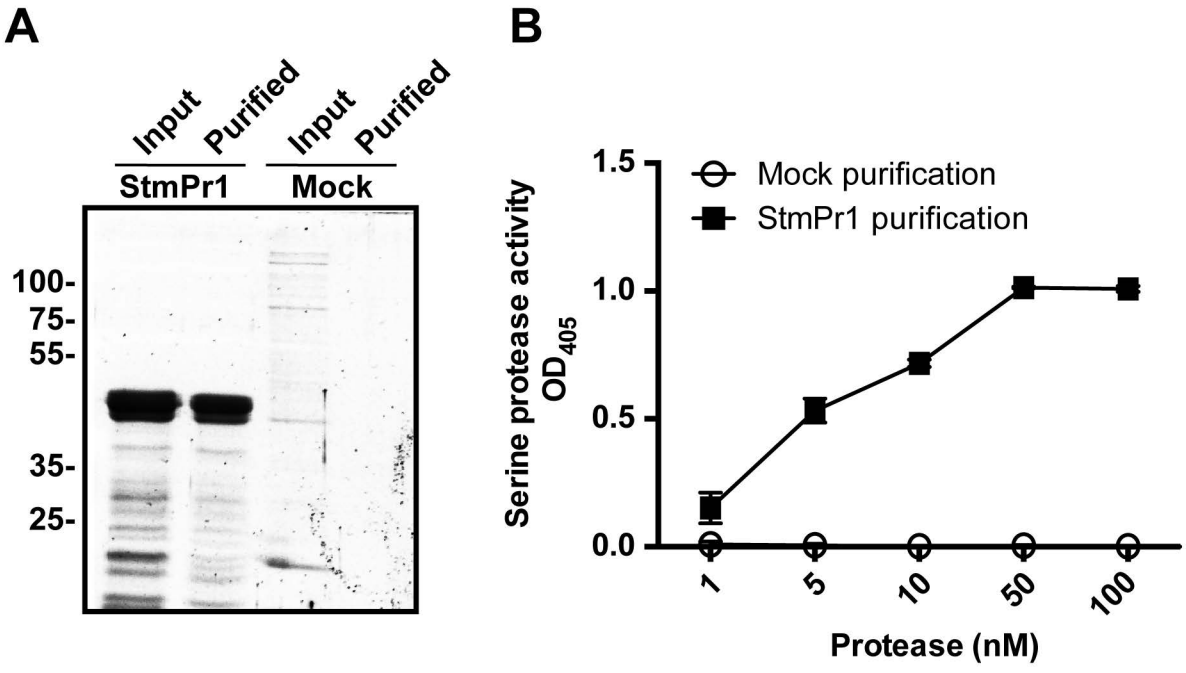

Fig. S3. Purification of StmPr1 from *S. maltophilia* culture supernatant. (A) StmPr1 was purified from the supernatant of the *stmPr1 stmPr2 stmPr3* mutant complemented with *stmPr1* using benzamidine sepharose, and analyzed by SDS-PAGE followed by SYPRO Ruby staining. A mock purification using supernatant from a *stmPr1 stmPr2 stmPr3* mutant transformed with an empty complementation vector was also performed. (B) Purified StmPr1 or mock purified material was incubated with N-succinyl-Ala-Ala-Pro-Phe-pNA at the indicated concentrations for 60 min at 37°C to evaluate serine protease activity. Purified StmPr1 was statistically different from the mock purified material at all doses, $P < .001$.

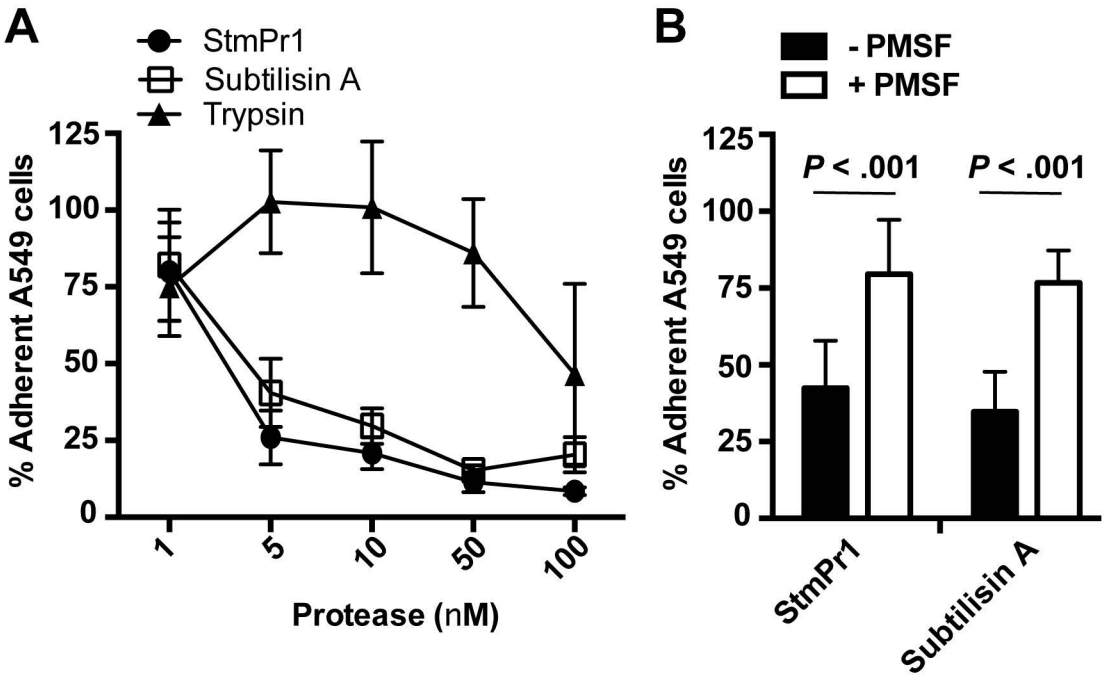

Fig. S4. Effect of a serine protease inhibitor on protease-mediated A549 cell detachment. (A) A549 cells were incubated for 3 h with the indicated equimolar concentrations of purified StmPr1, subtilisin A, or trypsin. (B) A549 cells were incubated for 3 h with 5 nM purified StmPr1 or subtilisin A in the presence or absence of the serine protease inhibitor PMSF. For (A and B), cell detachment was determined as described in Fig. 1. For (B and D), data are represented as the mean and SD of three independent experiments.

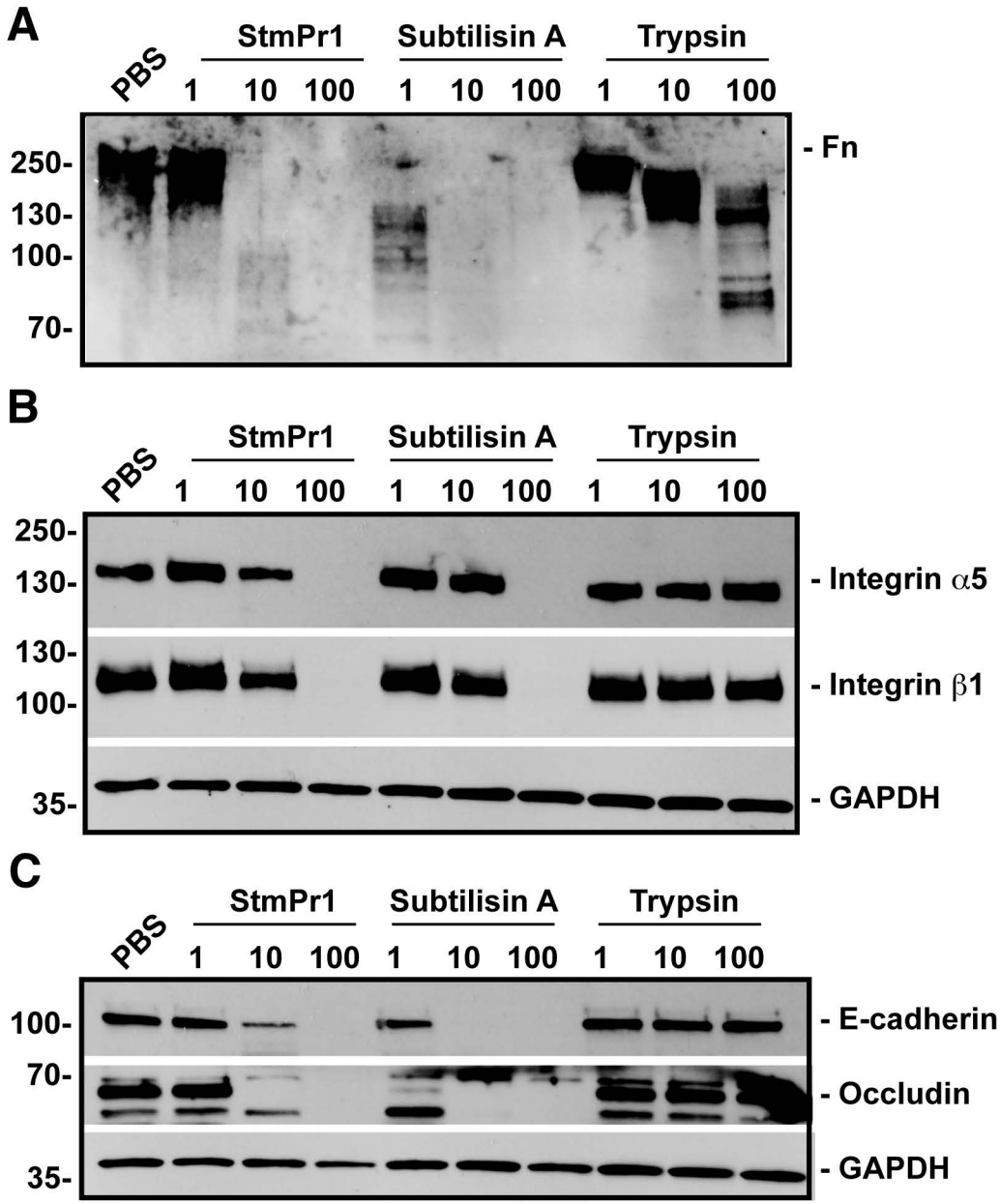

Fig. S5. Proteolytic degradation of adherence junction, tight junction, and ECM proteins during A549 culture. A549 cells were incubated for 1 h with the indicated equimolar concentrations of purified StmPr1, subtilisin A, and trypsin, as well as a PBS control. Cell culture supernatants (A) or cell lysates (B and C) were collected and analyzed by SDS-PAGE and immunoblot analysis with an anti-fibronectin (Fn) antibody (A); anti-integrin α5 and anti-integrin β1 antibodies (B); and anti-E-cadherin, and anti-occludin antibodies (C). For cell lysates (B and C), immunblot analysis with anti-GAPDH was also performed to confirm equal loading. Data are representative of three independent experiments.

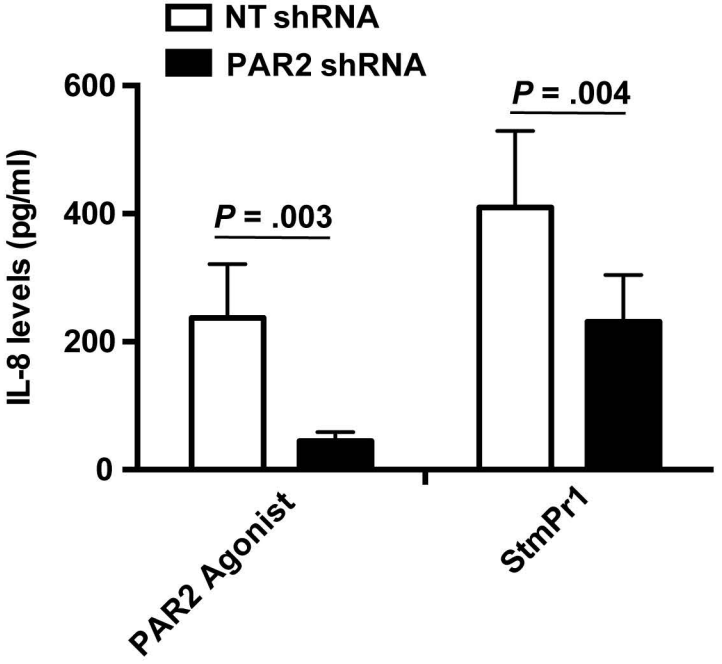

Fig. S6. Secreted IL-8 levels in NT control and PAR2 knockdown A549 cells stimulated with PAR2 agonist or StmPr1. A549 cells that were transfected with PAR2-targeting shRNA and non-targeting (NT) shRNA were incubated with 100 μM of the PAR2 agonist SLIGKV-NH2 and 3 nM purified StmPr1 for 16 h. IL-8 levels in the cell culture supernatants were quantified by ELISA.

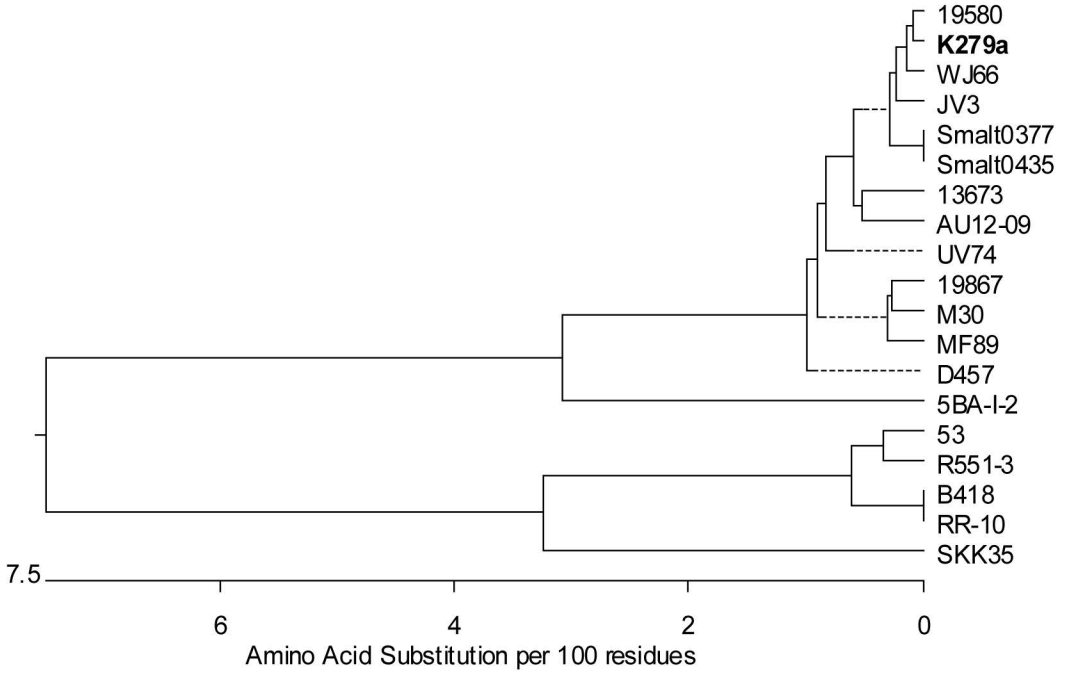

Fig. S7. Phylogenetic tree of *S. maltophilia* StmPr3 protein seqeunces. BLASTP analysis was performed using the StmPr3 sequences from strain K279a. StmPr3 aa sequences found in the BLASTP anlaysis were then obtained from NCBI, and aligned by Clustal W method using the MegAlign tool of DNASTAR Lasergene 9. Strain K279a is in bold.

Table S1 Primers used in this study

Primer	Sequence 5' to 3'[a]
AD94	AAACTCGAGGTGCCGAGGCCGCGATGG
AD95	AAAAAGCTTCGACTGCGCCTGTTCGCGC
AD86	AAAGTCCTGCCATGCCGATTCACAACAGAAGGATTCGTGTATGTGTGTAGGCTGGAGCTGCTTCG
AD87	AAAGGCGAGCGTGCTCAACAGAATGGTGGTCTTCATCATCACATATGAATATCCTCCTTA
AD129	AAAGAGCTCGTGCCGAGGCCGCGATGG
AD72	AAATGACGTACTAAGCGTTTGTGAC
AD73	AAACGGTAGCGGACGCCTTCAG
AD108	AAAGGGCCCGTCTTCATCTGCATGTGATTACC
AD112	AAAGAGCTCTTAGTGCCCCAGAACGCGGATC
SK251	AAAAAAGGGCCCGTTACCAGCGTGCGATCAG
SK252	AAAAAAGAGCTCTTACTGCGTGGCGAGAATG
AD01	AAAAAAGGGCCCGTCTGTGCAGGACACGAGAAG
AD02	AAAAAAGAGCTCTTA-GTACTGGGCGTTGAGGGTC
AD109	AAAGAGCTCTTAGTGATGGTGGTGATGGTGGTGCCCCAGAACGCGGATC

[a] Restriction sites are underlined