Hamilton et al., http://www.jcb.org/cgi/content/full/jcb.201404127/DC1

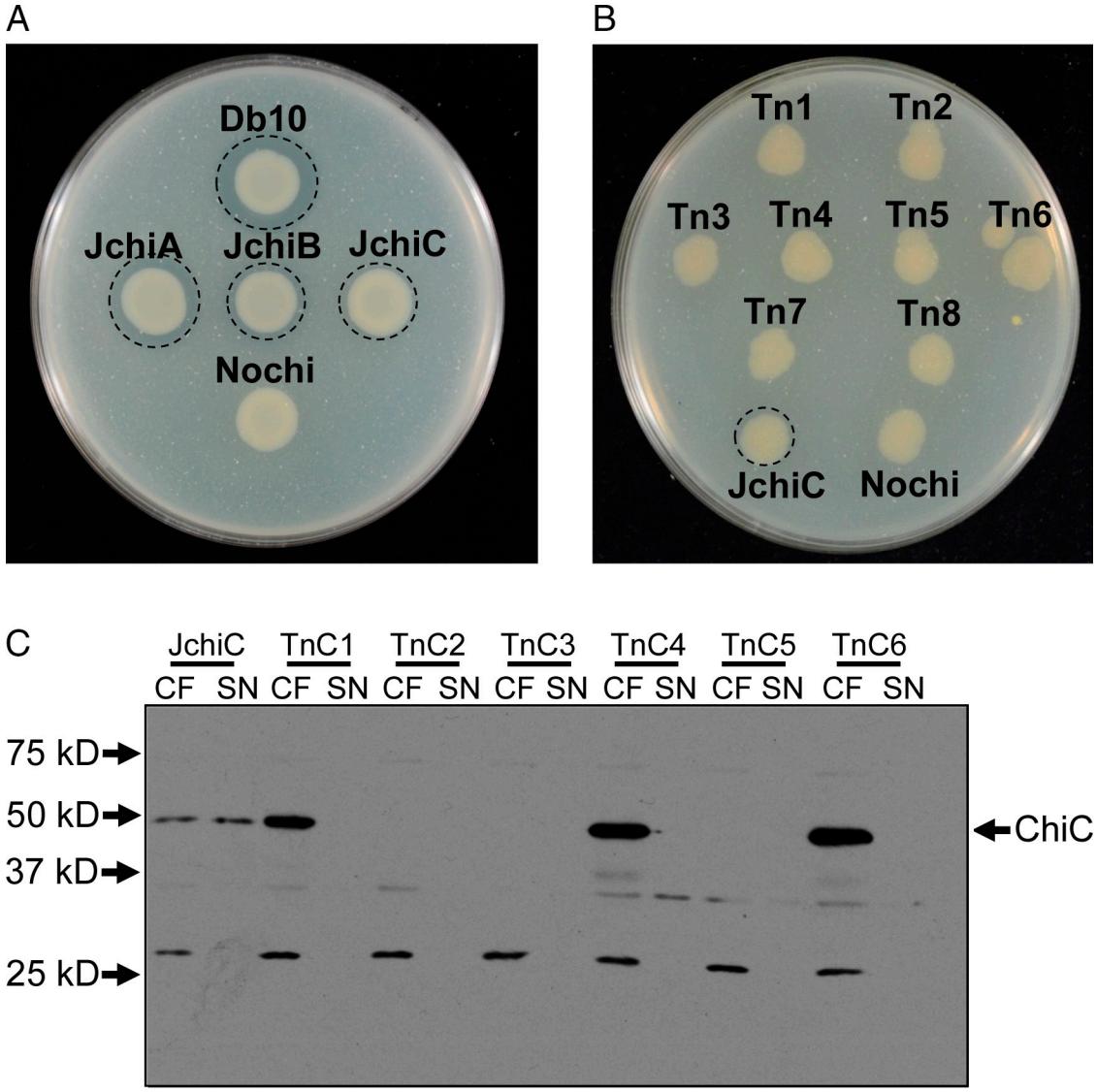


Figure S1. **Directed and transposon mutagenesis identifies genes required for in vivo chitinolytic activity by *S. marcescens*.** LB-agar plates were prepared supplemented with 2% (wt/vol) colloidal chitin. Liquid precultures of *S. marcescens* strains were then spotted on and incubated overnight at 30°C. (A) A typical chitinolytic assay featuring a positive control (Db10), a negative control (Nochi), and strains producing ChiA only (JchiA), ChiB only (JchiB), and ChiC only (JchiC). The dotted lines mark pout the zones of clearing observed. A large zone of clearing is observable around Db10, with lesser zones around the single chitinase mutants. The ChiC only strain has the smallest zone of clearing. The triple mutant (Nochi) is unable to produce a zone of clearing. (B) Transposon insertion mutants (Tn1–Tn8) isolated after mutagenesis of the JchiC strain. (C) The JchiC strain together with six of the isolated transposon mutants defective in chitinolytic activity were grown aerobically in liquid culture. Culture supernatants (SN) were separated from whole cell fractions (CF), and the proteins were separated by SDS-PAGE and analyzed for the presence of ChiC by Western immunoblotting.

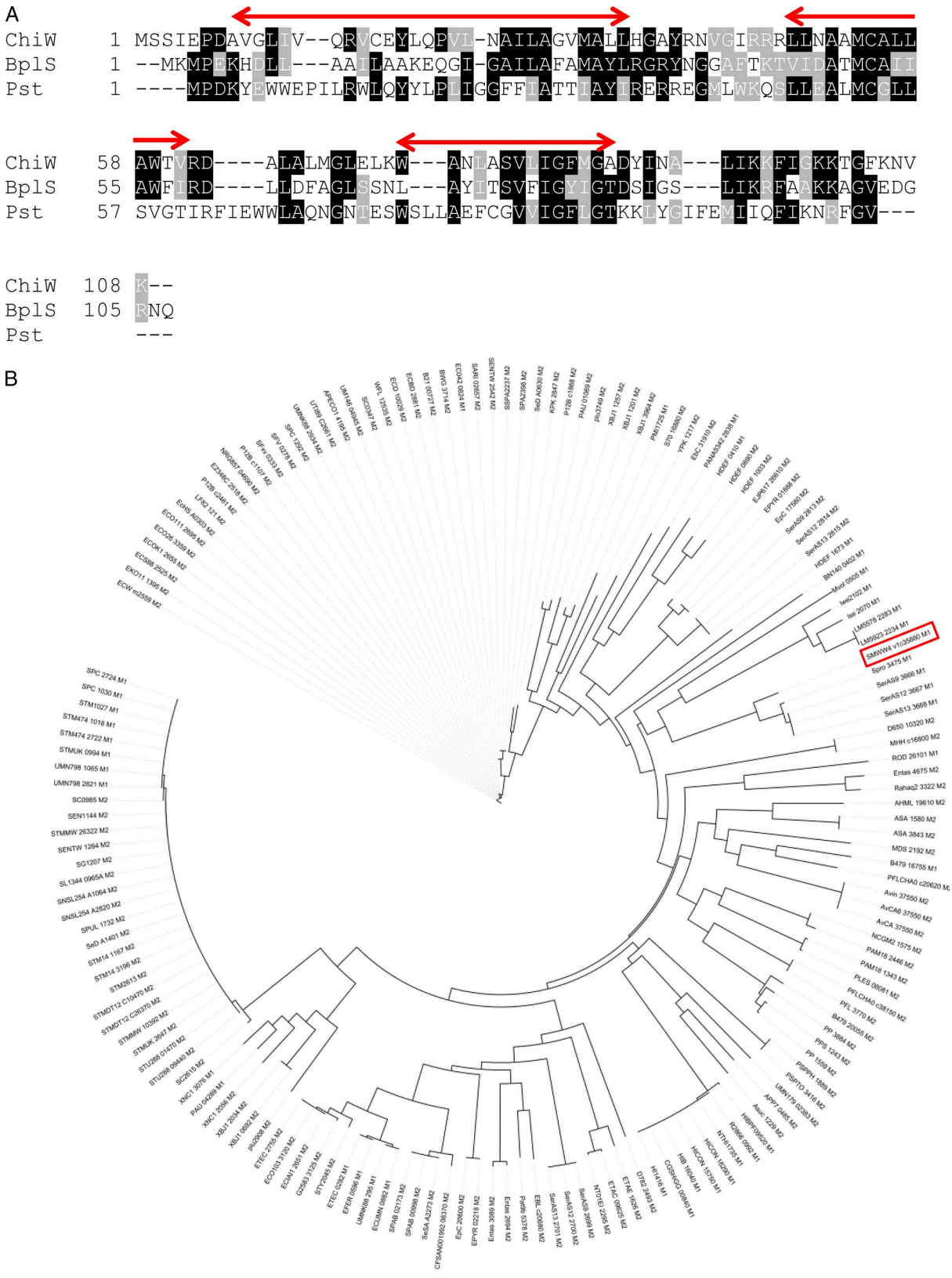


Figure S2. **The ChiW protein and its homologues.** (A) An alignment of *S. marcescens* ChiW with the canonical λ S107 holin (BplS) together with a bacterial genome-encoded holin-like protein that displays, like ChiW, a single translation initiation codon. This is the *Providencia stuartii* ATCC25827 PROSTU_03068 protein (Pst). The determined positions of the three λ S holin transmembrane domains are shown by red arrows. Black shading highlights identical amino acid side chains, whereas gray shading highlights similar amino acid residues, at a given position in the sequences. (B) All putative holins identified that were 130 residues or less (i.e., 40 with a single N-terminal methionine [labeled M1] and 121 with a second N-terminal methionine [labeled M2]) were aligned using the T-Coffee program at https://www.ebi.ac.uk/Tools/msa/tcoffee/. The Interactive Tree of Life website http://itol.embl.de/index.shtml was used to generate the phylogenetic tree. The location of the *S. marcescens* ChiW sequence is highlighted by the red box.

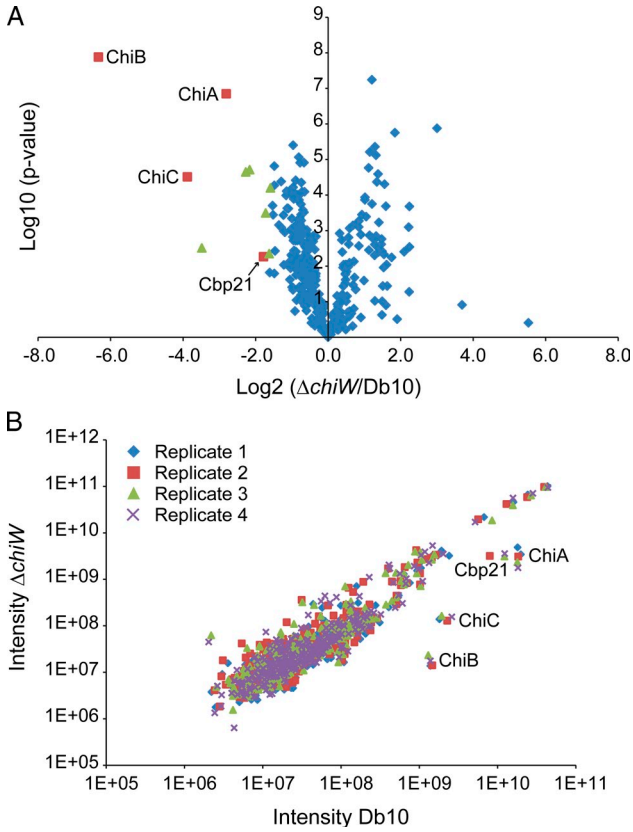


Figure S3. **Proteomics quality control.** (A) Volcano plot of the proteomics experiment shows an expected tight distribution for the majority of proteins with the few significantly reduced proteins in the JJH08p Δ*chiW* strain (Δ*chiW*) mutant colored green and red. (B) An intensity scatter plot of all four biological replicates shows the high reproducibility of the changing chitinases and the chitin-binding protein Cbp21.

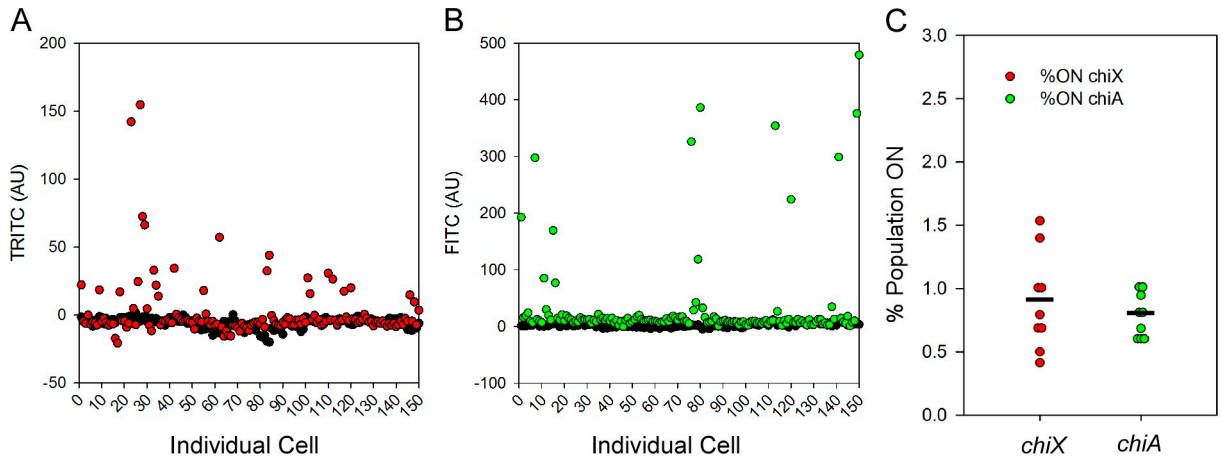
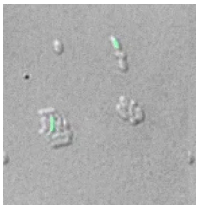


Figure S4. **The expressions of the *chiX* and *chiA* genes exhibit a bimodal distribution.** (A) Representative data for *chiX* expression as followed by mKate fluorescence. The background level in the TRITC channel was calculated as the mean value of *S. marcescens* Db10 parent strain grown under the same conditions, plus two times the standard deviation. The background value was set at 0 on the x axis. Those JJH09 (Δ*chiX*::mKate) cells defined as ON cells exhibited fluorescence well above the background value. 150 cells are represented for the both the parental Db10 strain (black circles) and Δ*chiX*::mKate strain (red circles). The Δ*chiX*::mKate cells were chosen to represent the range of expression values observed. (B) Representative data for *chiA* expression as followed via GFP fluorescence. The background level in the FITC channel was calculated as the mean value of *S. marcescens* Db10 parent strain grown under the same conditions, plus two times the standard deviation. The background value was set at 0 on the x axis. Those *chiA*::*gfp* cells defined as ON exhibited fluorescence above the background value. 150 cells are represented for both the parental Db10 strain (black circles) and *chiA*::*gfp* strain (green circles). (C) The percentage of mKate2- or GFP-positive cells per field of view was calculated for nine independent images from two independent experiments. The percentage of the population that was ON is plotted for both strains. The black bars represent the mean values. AU, arbitrary unit.

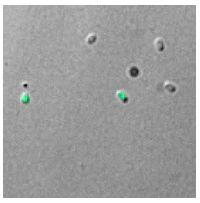
Table S1. **Extracellular proteins of increased abundance in a ChiW-deficient mutant of *S. marcescens* Db10 identified by label-free mass spectrometry**

Identifier	Name	Sec signal peptide	Description	Intensity	Abundance Δ*chiW*/Db10	P-value	Unique peptides	Sequence coverage
								%
SMDB11_3915	FimA	Yes	Type 1 fimbrin	4.1×10^{7}	8.0	1.33×10^{-6}	6	60
SMDB11_1250	FimI	Yes	Type 1 fimbrin	1.6×10^{8}	4.7	0.0028	3	34
SMDB11_4526	RplU	No	50S ribosomal protein L21	6.7×10^{6}	4.7	0.0002	3	26
SMDB11_0789	–	Yes	Predicted fimbrial-like adhesion protein	6.5×10^{7}	4.7	0.0008	4	35
SMDB11_1052	SafA	Yes	Mannose-sensitive type 1 fimbrin	3.4×10^{8}	4.3	0.0039	8	70
SMDB11_3920	PmfE	Yes	Fimbrial adhesin	1.0×10^{7}	3.6	1.77×10^{-6}	7	27
SMDB11_2217	FlgD	No	Flagellar assembly protein	3.4×10^{7}	3.4	0.0057	4	32
SMDB11_4500	RplI	No	50S ribosomal protein L9	4.2×10^{6}	3.1	0.0091	4	30
SMDB11_2263	Hcp	No	Type VI component/ secreted protein	1.5×10^{10}	3.0	0.0002	19	92

Proteins identified as present in the secretome of a ChiW-deficient mutant (JJH08p) at an abundance greater than 3× higher than observed in the secretome of the parent strain Db10 ($P < 0.01$) are shown. Four biological replicates of each strain were analyzed. Minus signs indicate that this gene product has no name.



Video 1. **Fate of strain FTG005 GFP-positive cells upon time-lapse imaging—dividing cell.** Strain FTG005 (ϕ*chiA*::*gfp*) was grown for 18 h in minimal media before being diluted and applied to a microscope slide. Images were analyzed by time-lapse epifluorescence microscopy using a wide-field microscope (DeltaVision Core; Applied Precision) mounted on an inverted stand (IX71; Olympus) with a 60×, 1.4 NA lens (Olympus) and a camera (CoolSNAP HQ; Photometrics) with DIC and fluorescence optics. The left-hand image is the merge of the DIC and FITC channel (false-colored green), the center image is the DIC channel, and the right-hand image is the FITC channel (shown in white). Frames were taken every 15 min for 4 h and 30 min and are at four frames per second for display.



Video 2. **Fate of strain FTG005 GFP-positive cells upon time-lapse imaging—quiescent cell.** Strain FTG005 (ϕ*chiA*::*gfp*) was grown for 18 h in minimal media before being diluted and applied to a microscope slide. Images were analyzed by time-lapse epifluorescence microscopy using a wide-field microscope (DeltaVision Core; Applied Precision) mounted on an inverted stand (IX71; Olympus) with a 60×, 1.4 NA lens (Olympus) and a camera (CoolSNAP HQ; Photometrics) with DIC and fluorescence optics. The left-hand image is the merge of the DIC and FITC channel (false-colored green), the center image is the DIC channel, and the right-hand image is the FITC channel (shown in white). Frames were taken every 15 min for 4 h and 30 min and are at four frames per second for display.