Supplementary methods

Detailed plasmid construction

Plasmids generated in previous work are listed in table S1.

To obtain p*cts2Δ*_hyg, we PCR amplified a 1.6 kb upstream part of *cts2* with the primers RL495/RL496 and a downstream part with RL497/RL498 integrating BsaI-restriction sites for Golden Gate cloning. The flanks together with the hygromycin resistance cassette from pStorI_1hs were cloned into the plasmid backbone of pDest. p*cts3Δ*_hyg was obtained by integrating a PCR generated 1.5 kB fragment (upstream of the *cts3* gene; with primers RL479/480) and a 900 bp downstream fragment (with primers RL481/482) into pRS426 via yeast recombinational cloning. p*cts2Δ*_nat and p*cts3Δ* _g418 were obtained by replacing the hygromycin resistance cassette by the nourseothricin resistance cassette from p*rrm4Δ*_nat or the G418 resistance cassette from pMFg1, respectively. To obtain p*cts4Δ*_hyg upstream and downstream flanking regions of the *cts4* ORF were PCR generated using the primer pairs RL1267/RL1268 and RL1269/RL1270, respectively, and cloned into the plasmid backbone of pDest together with the hygromycin resistance cassette of pStorI_1hs. p*cts4Δ*_cbx was obtained by replacing the hygromycin resistance cassette from p*cts4Δ*_hyg by the carboxin resistance cassette from pStorI_1c. To obtain p*cts2:egfp* the upstream flank, containing the *cts2* ORF, and the downstream flank were generated by PCR using the primer pairs RL1292/RL1293 and RL1294/RL1295, respectively, and cloned into pDest together with the hygromycin resistance cassette of pStorII_5-1h. Similarly, p*cts3:egfp* was obtained by cloning PCR generated flanks (primer pairs RL1300/RL1301 and RL1302/RL1303) with the same vectors.

Mating assay

To test mating competence of *U. maydis* chitinase mutants, cultures were grown to an OD_{600} of 0.8 in CM, washed once in H_2O and resuspended to an OD_{600} of 1 in H_2O. 500 µl of the suspension of each mating partner was premixed in a reaction tube. 60 µl of the mating reaction was spotted into an empty petri dish, sealed with parafilm and incubated O/N at 22 °C. Mating was observed microscopically.

RNA isolation, generation of cDNA, and semi-quantitative PCR

RNA was extracted from yeast cultures similar to (1) using Trizol (Invitrogen) according to the manufacture's instructions. DNA contaminations were removed by treatment with Turbo-DNase (Ambion). After DNase treatment total RNA was purified by acidic phenole/chloroform purification. 1 µg of total RNA was used for reverse transcription with Superscript III First-Strand Synthesis SuperMix (Invitrogen) and Oligo$(dT)_{20}$ primers according to manufacturer's instructions. For semi-quantitative RT-PCR 1 µl cDNA (1 µg/µl) was used in a PCR-reaction undergoing 25 cycles (logarithmic phase of amplification). Three independent experiments were conducted with biological replicates each leading to reproducible results. Specific primers for cts1, cts2, cts3, and cts4 were used to determine relative gene expression. Actin specific primers were used as an endogenous control for each template. cDNA from yeast cells and filaments were used to determine gene expression patterns in each growth phase. Minus reverse transcriptase controls were added to exclude contamination by genomic DNA. Genomic DNA was used as template to determine primer efficiency and H_2O was used as negative control.

Supplementary tables

Table S1: Plasmids used in this study

Plasmid	Reference
p*rrm4Δ* _nat	(2)
pMF1g	(3)
pRS426	(4)
pDest	(5)
pStorI_1hs	(5)
pStorII_5-1h	(5)
p*cts2Δ* _hyg	this study
p*cts3Δ* _hyg	this study
pStorI_1c	(5)
p*cts2Δ* _nat	this study
p*cts3Δ* _g418	this study
p*cts4Δ* _hyg	this study
p*cts2:egfp*	this study
p*cts3:egfp*	this study
p*cts4Δ* _cbx	this study

Table S2: DNA oligonucleotides used in this study

Designation	Sequence
MF502	ACGACGTTGTAAAACGACGGCCAG
MF503	TTCACACAGGAAACAGCTATGACC
MF963	TGGAAAGCGGGCAGTGAG
MF964	GCGCAACTGTTGGGAAGG
RL479 cts3 U2	GTAACGCCAGGGTTTTCCCAGTCACGACGAATATTACATCAGCTATTCAGTGTTACGC
RL480 cts3 U3	CCACTCGAGTGCGGCCGCAATTGTCACGCCATGGTTAACTCCTAATGTGTGCG
RL481 cts3 D1	CTGCTGCGCCGCGAAGCTGTGCGGCCGCATTAATAGGTCTGCGCACCTGTTTGG
RL482 cts3 D2	GCGGATAACAATTTCACACAGGAAACAGCAATATTTCCACACCTTAGTGACGG
RL483 cts3 P1	GAAGCTTCTCCCACTCGG
RL484 cts3 P2	GTTGACGCTCTTGGTCGG
RL485 cts3 U1	TTCCATGCCACGCACACC
RL486 cts3 D3	CAAGCACAGACGGTTCGC
RL495 cts2 U2	GGTCTCGCCTGCAAATATTTCGTAATTCGTGATGG
RL496 cts2 U3	GGTCTCCAGGCCCGCGGTGACCGAGAGGGG
RL497 cts2 D1	GGTCTCCGGCCATACGCTATCACACATCC
RL498 cts2 D2	GGTCTCGCTGCAAATATTTGGTGACTGTCAATACC
RL499 cts2 P1	GTCACGCACTCAGTGTGG
RL500 cts2 P2	GGCAGTTGGTTTCGCAGC
RL501 cts2 U1	TGTGTACACACTGGCGCG
RL502 cts2 D3	TAGCACCGATTGCGAGCG
RL955_cts2_probe-for	GCTGACACGAGAATCGTG
RL956_cts2_probe-rev	GCCAAGCGTACAGACCTAC
RL957_cts2_Probe-DF-for	CCATCTCAACCGCCTTTC
RL958_cts2_Probe-DF_rev	GGCAAACTCACACGGAAG
RL959_cts3-Probe-UF-for	GATGCGAACCTCAGGCATC
RL960_cts3-Probe-UF-rev	CGTATGAGCACATGCTACG
RL961_cts3-Probe-DF-for	GTTGGTCTTGGCTCTCAC
RL962_cts3-Probe-DF-rev	CATCCACGCCTTGGACAC
RL1266_U1 cts4	TTTGGTGCCTCGACTCGC
RL1267_U2 cts4	GGTCTCGCCTGCAATATTCGCGACTCGACCTTTTTAC
RL1268_U3 cts4	GGTCTCCAGGCCTGTTTGTGAAGCAGGTATG
RL1269_D1 cts4	GGTCTCCGGCCCGACACAGCACTCGCCGTG
RL1270_D2 cts4	GGTCTCGCTGCAATATTCATGCGTCCGCTTCAAG
RL1271_D3 cts4	GCTACTGACCTTGTTGCC
RL1272_P1 cts4	TCGTTGGCGCTTTCTCTG
RL1273_P2 cts4	AGGGCAAGGAGTTGTGTC
RL1291_U1-cts2	ACAACGCGCAGAAGTTCG
RL1292_U2-cts2	GGTCTCGCCTGCAATATTGATGGTTTCCGTGTTTGG
RL1293_U3-cts2	GGTCTCCTGGCCATGCTCAATCCGGCAGCGTC
RL1294_D1-cts2	GGTCTCCGGCCACAGCATACGCTATCACAC
RL1295_D2-cts2	GGTCTCGCTGCAATATTCTGTGTTCTCAATGCCCGGC
RL1296_D3-cts2	CGCGATCTGCTTGAACATCC
RL1297_P1-cts2	ACAAGTTGGTCGTCGGTAAG
RL1298_P2-cts2	TTGAAAGGCGGTTGAGATGG

Table S2 (continued): DNA oligonucleotides used in this study

RL1299_U1-cts3	GCCTACAGCCGAGTTTGATC
RL1300_U2-cts3	GGTCTCGCCTGCAATATTCGTCAAGGCAGCCAAG
RL1301_U3-cts3	GGTCTCCTGGCCAGGGAAGAGATAGCACCTGTC
RL1302_D2-cts3	GGTCTCCGGCCGTCTGCGCACCTGTTTGGTGTTG
RL1303_D2-cts3	GGTCTCGCTGCAATATTCCAACAGGATCCTACG
RL1304_D3-cts3	CACAAACTCATCCACTCCCG
RL1305_P1-cts3	TCAACGCTCGTCTCACAG
RL1306_P2-cts3	TAGCTAGCACGCATACAG
RL1314_cts3-seq-f	GGATTGGACAGCCTCGAC

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4. **Sikorski RS, Hieter P.** 1989. A System of Shuttle Vectors and Yeast Host Strains Designed for Efficient Manipulation of DNA in Saccharomyces-Cerevisiae. Genetics **122:**19-27.
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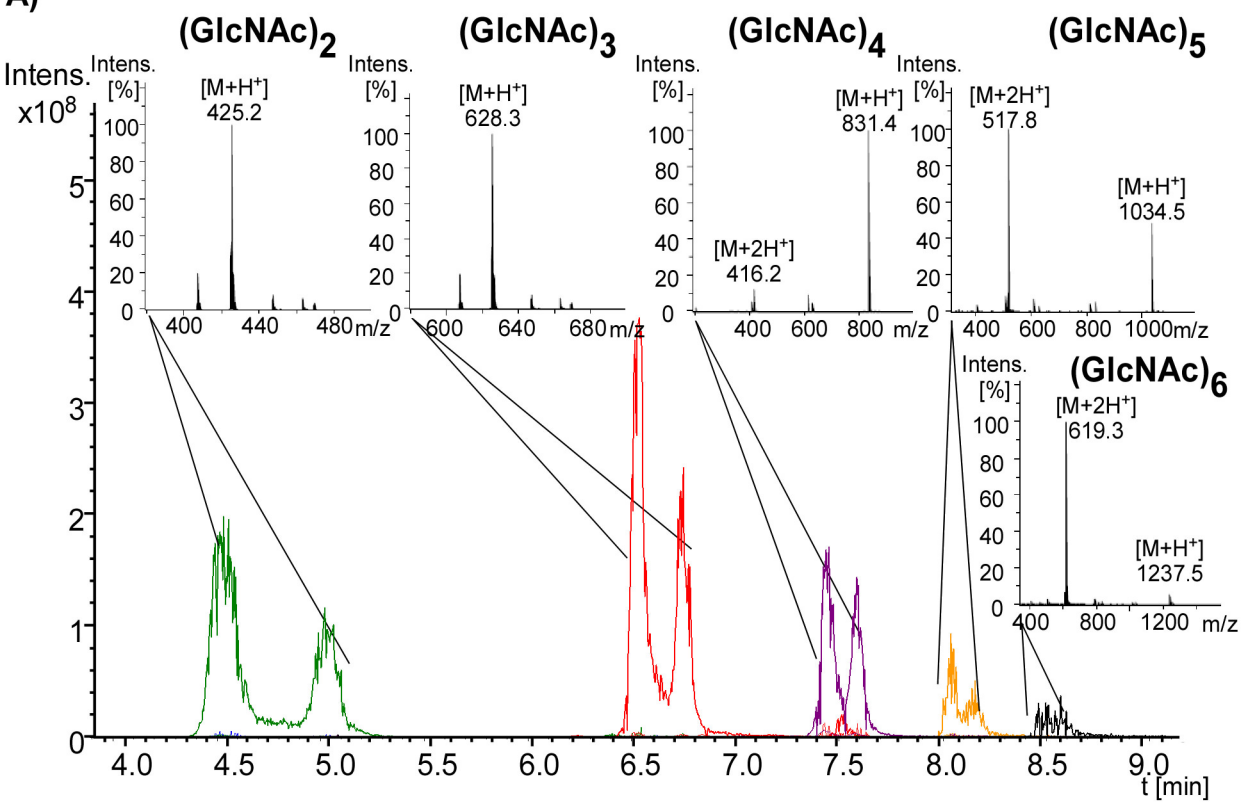
A)



B)

Product	Conjugated ion				
		H^+	$2\ H^+$	$3\ H^+$	Na^+
GlcNAc	221.1	222.1	111.5	74.7	244.1
$(GlcNAc)_2$	424.2	425.2	213.1	142.4	447.2
$(GlcNAc)_3$	627.2	628.2	314.6	210.1	650.2
$(GlcNAc)_4$	830.3	831.3	416.2	277.8	853.3
$(GlcNAc)_5$	1 033.4	1 034.4	517.7	345.5	1 056.4
$(GlcNAc)_6$	1 236.5	1 237.5	619.2	413.2	1 259.5

Fig. S1: Chito-oligosaccharide standards for UPLC-MS analysis of hydrolysis products. (A) Extracted Ion Chromatogram (EIC) and ESI-MS analysis of specific chito-oligosaccharides). $(GlcNAc)_2$ (m/z 425.2; green), $(GlcNAc)_3$ (m/z 628.3; red), $(GlcNAc)_4$ (m/z 831.4; purple), $(GlcNAc)_5$ (m/z 517.7; yellow) and $(GlcNAc)_6$ (m/z 619.3; black). **(B)** Theoretical masses of chito-oligosaccharides with different charges and adducts used for the EIC spectra.

Figure S2

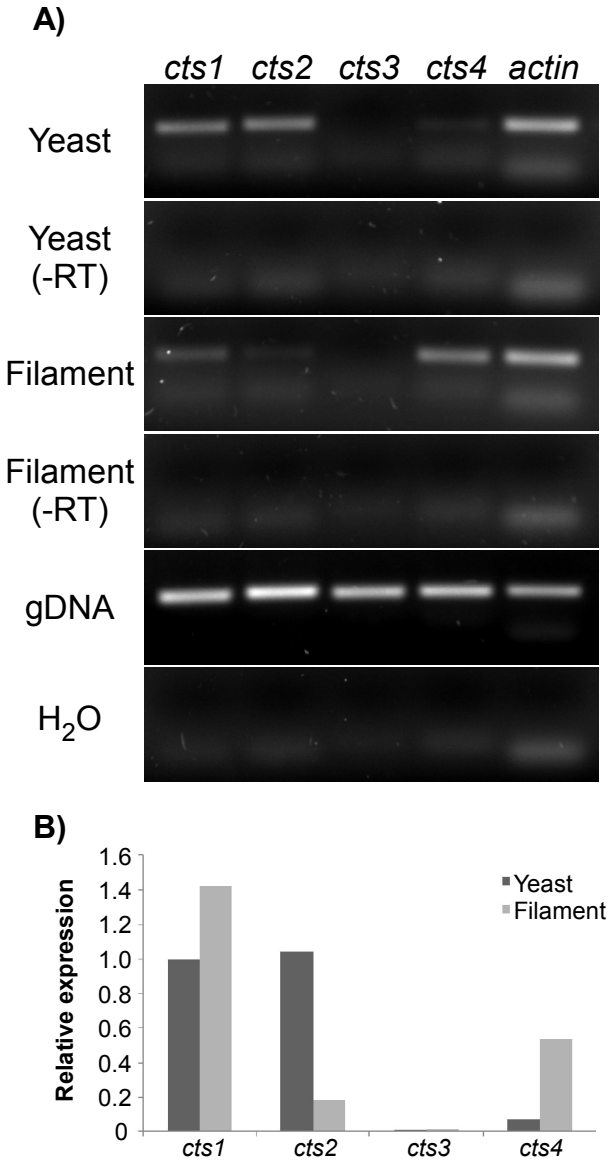


Fig. S2: Gene expression analysis of chitinolytic enzymes. (A) Semi-quantitative RT-PCR of chitinase genes in yeast cells and filaments. Both, *cts1* and *cts2* are expressed equally strong during yeast-like growth of AB33. During filamentous growth *cts2* gets drastically down regulated. *cts3* is not expressed in either yeast cells or filaments. *cts4* shows basal expression in yeast cells and gets up regulated in filaments. *actin* was included as an endogenous control. Minus reverse transcriptase (-RT) controls of each sample were used to exclude contamination by genomic DNA. **(B)** Similar expression patterns for *cts1*, *cts2*, *cts3*, and *cts4* were extracted from published microarray data (Heimel et al., 2010).

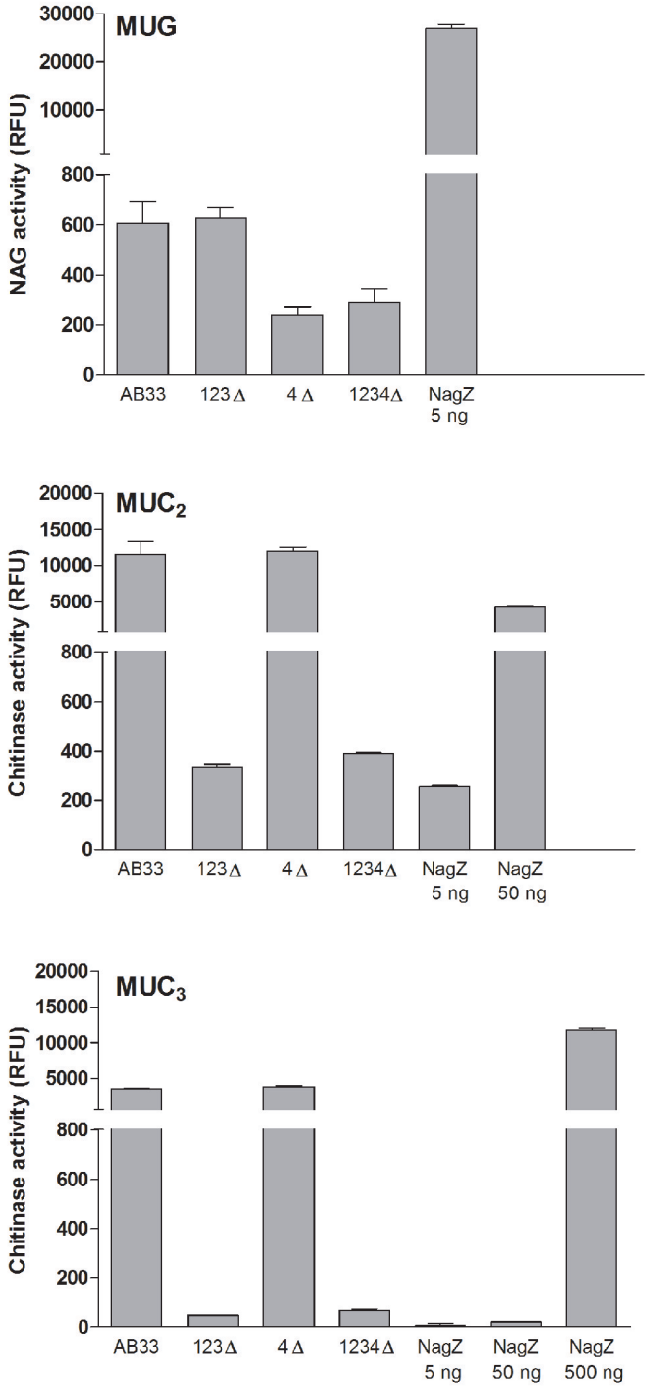


Fig. S3: NAG activity towards the chitinase substrates MUC_2 and MUC_3. To exclude contribution of Cts4 NAG-activity towards fluorescent chitinase substrates, intact cells of AB33, AB33cts1Δcts2Δcts3Δ, AB33cts4Δ, and AB33cts1Δcts2Δcts3Δcts4Δ were compared to BsNagZ (N-acetyl-glucosaminidase nagZ from *Bacillus subtilis*; Hamer et al., 2014). 5 ng NagZ efficiently degrade MUG, but 10x higher concentrations are required for degradation of MUC_2 and 100x higher concentrations for MUC_3. Activity of Cts4 in AB33cts1Δcts2Δcts3Δ towards MUG is lower than the activity of 5 ng NagZ and is at the same basal level below the detection limit in assays with the MUC_2 and MUC_3. Therefore, low levels of Cts4 potentially influencing chitinase activity measurements in this assay is neglectable.

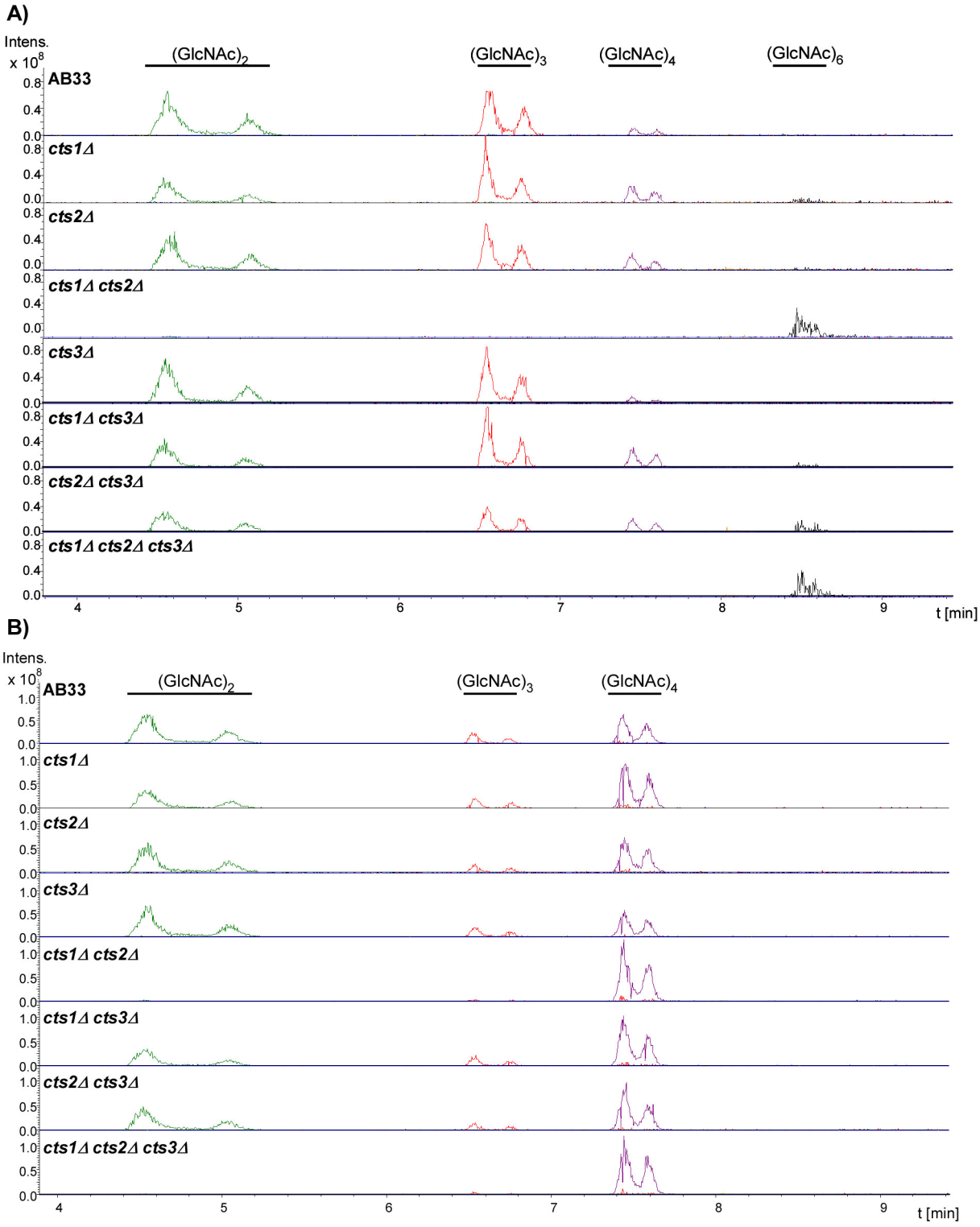


Fig. S4: Cts1 and Cts2 process chito-oligosaccharides. Extracted Ion Chromatograms (EIC) of hydrolysis products of chito-oligomers incubated with 5 µg *U. maydis* total protein extracts. **(A)** The chito-hexamer $(GlcNAc)_6$ is partially degraded in strains containing either Cts1 or Cts2, but not in double mutants lacking both chitinases. **(B)** Similarly, the chito-tetramer (GlcNAc)4 is degraded in strains containing Cts1 and Cts2, but not in the double mutants. $(GlcNAc)_2$ (m/z 425.2; green), $(GlcNAc)_3$ (m/z 628.3; red), $(GlcNAc)_4$ (m/z 831.4; purple) and $(GlcNAc)_6$ (m/z 619.3; black).

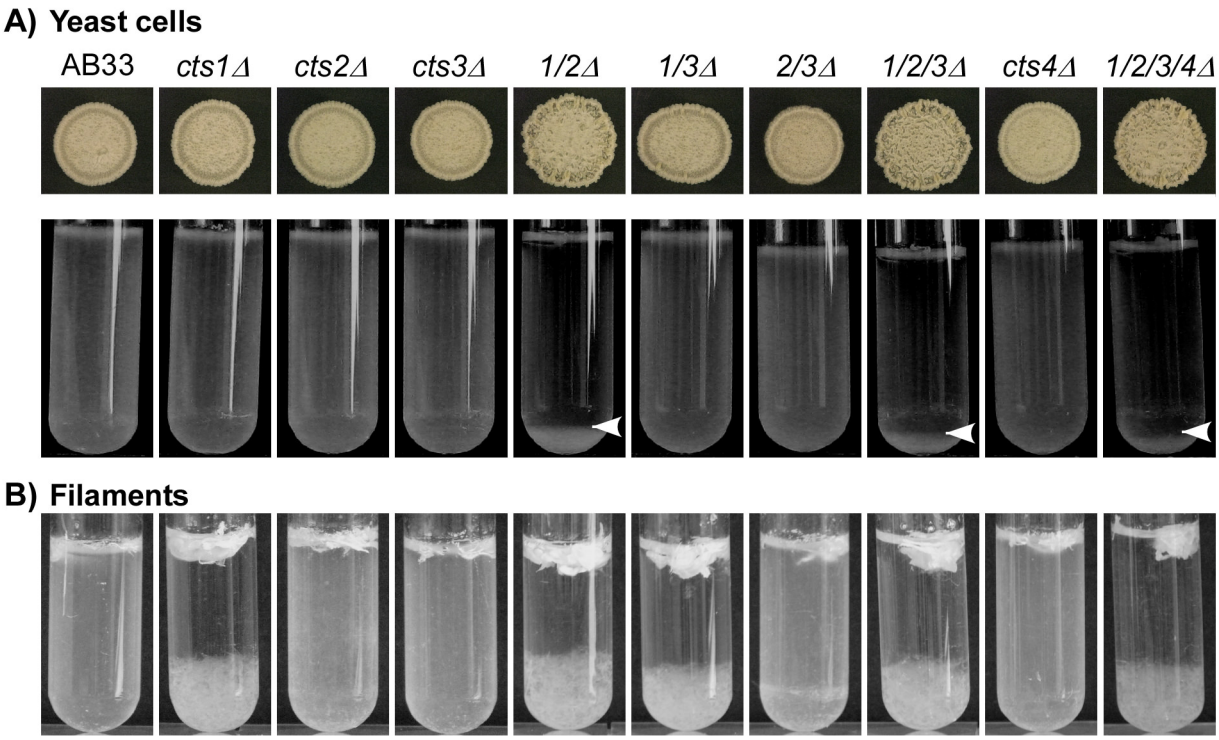


Fig. S5: Macroscopic phenotypes of the chitinase deficient mutants. (A) Colony morphology and sedimentation assay of *U. maydis* chitinase deletion strains. *cts1/2Δ*– deletion strains and derived mutants show an altered colony morphology and form aggregates which sediment fast in liquid medium as indicated by the arrowhead. **(B)** Sedimentation assay of AB33 and chitinase deficient strains. Strains were grown to an OD_{600} of 0.5 and transferred to NM-G for 16h for filament induction. After 5 min without shaking all strains which lack the chitinase Cts1 showed faster sedimentation in liquid medium.

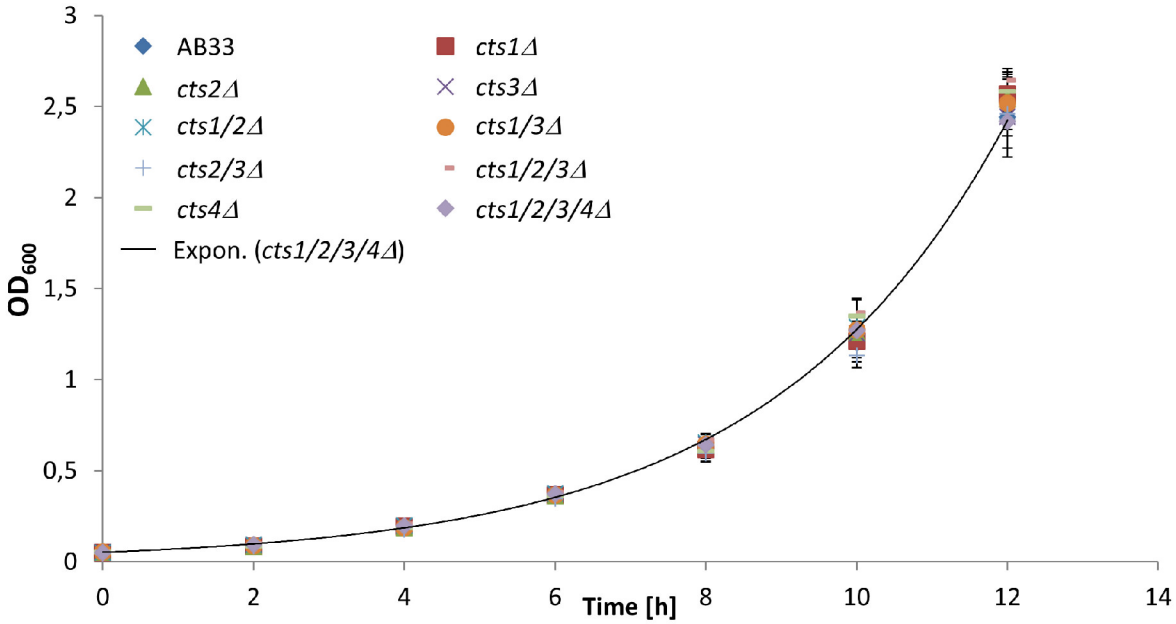


Fig. S6: Chitinases do not affect growth rate of *U. maydis*. Exponentially growing cultures at an OD_{600} of 0.8 were diluted to an OD_{600} of 0.05 and growth was measured as OD_{600}. All mutants up to the complete chitinase deficient quadruple mutant have doubling times comparable to AB33. The line shows an exemplary exponential fit for AB33cts1Δcts2Δcts3Δcts4Δ illustrating exponential growth. Error bars represent standard deviation from 3 independent experiments.

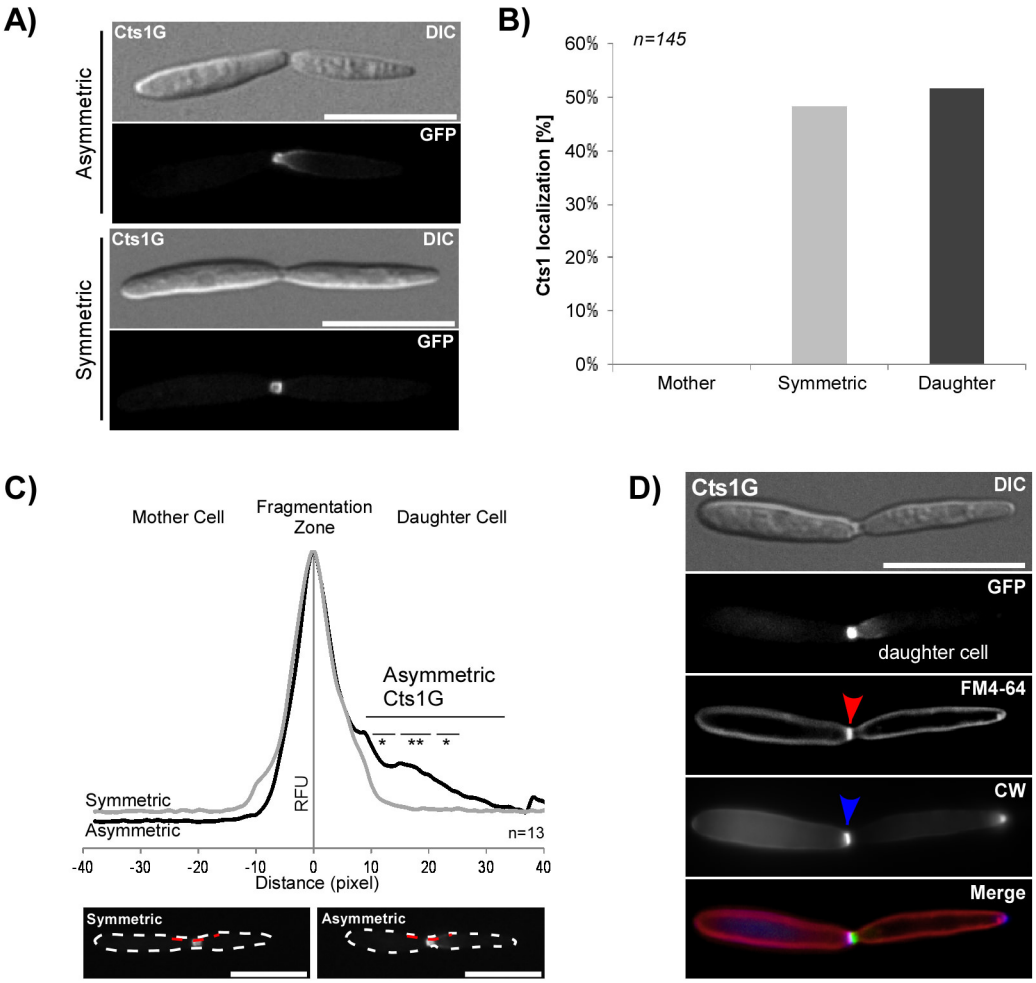


Fig. S7: Cts1G localizes to the fragmentation zone from the daughter cell. (A) Fluorescent microscopic observation of Cts1G localization. Cts1G localizes either asymmetrically forming a gradient with a maximum at the fragmentation zone (top) or symmetrically to the center of the fragmentation (bottom). **(B)** Quantification of Cts1G localization. A total of 145 cells were observed and localization was categorized as "Mother" (Cts1G localization at the mother side), "Symmetric" (Cts1G localization to the center of the fragmentation zone), or "Daughter" (Cts1G localization to the daughter side). 70 cells (48.3 %) showed symmetric Cts1G, 75 cells (51.7 %) showed Cts1G localization to the daughter side, and no cells showed Cts1G localization to the mother side. **(C)** Linescan analysis of asymmetric Cts1G distribution. A portion of Cts1G localizes asymmetrically at the daughter side, whereas the amount of cytoplasmic Cts1G is similar in all cells. A total of 26 cells (13 symmetric, 13 asymmetric) were analyzed by linescan as shown exemplarily (bottom panel, red line). Cts1G is significantly enriched at the daughter side. *, $p<0,05$, **, $p<0,005$ (students t-test). **(D)** Fluorescent co-localization analysis of asymmetric Cts1G localization with the lipophilic dye FM4-64 and the cell wall marker CW. Cts1G localizes to the fragmentation zone after primary septum formation by the mother cell. Cell membrane: red arrowhead; primary septum: blue arrowhead.

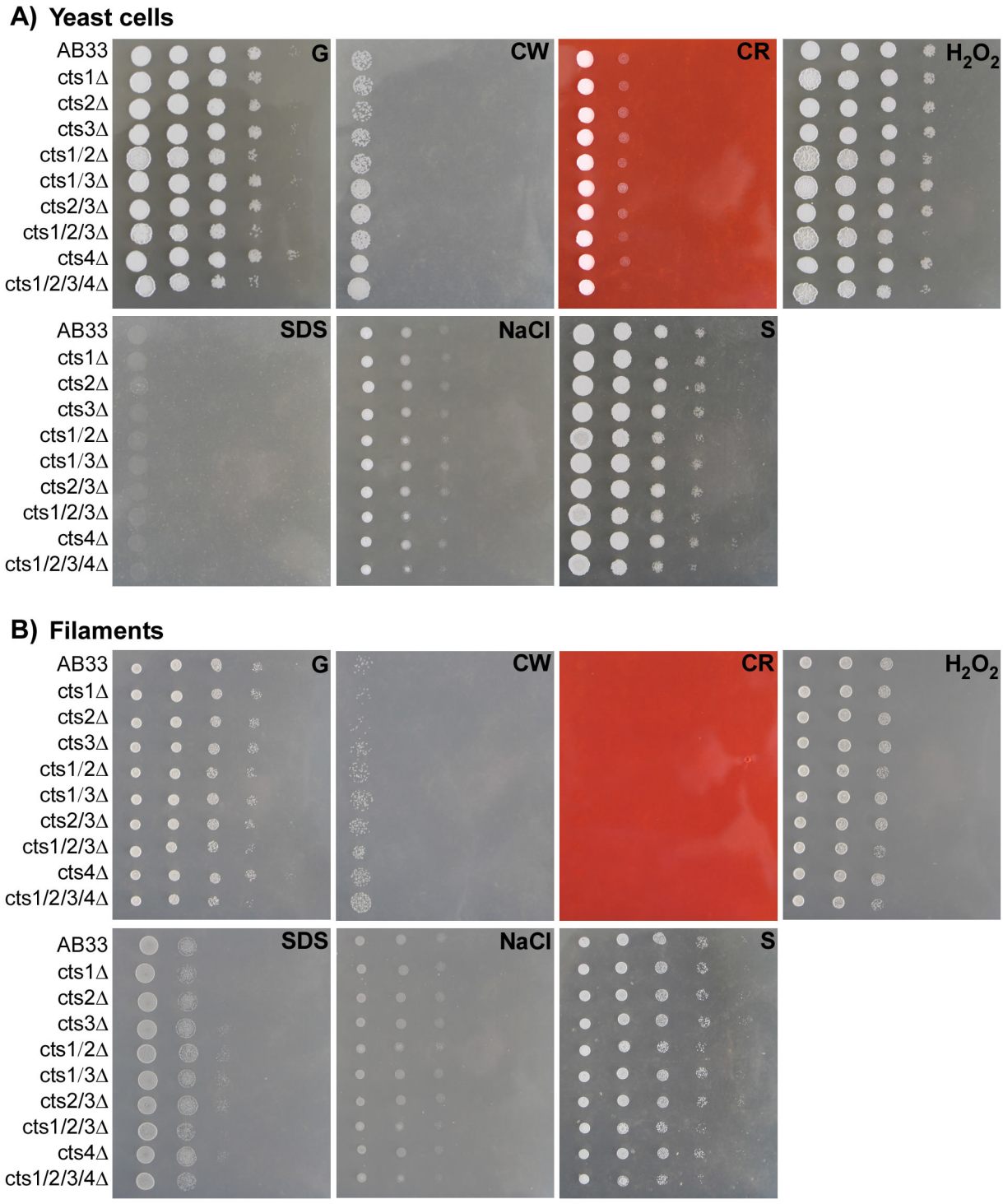


Fig. S8: Chitinases do not influence stress tolerance of *U. maydis*. Plate growth assay of *U. maydis* strains on CM-G (yeast-cells) and NM-G (filaments) agar plates containing the stress inducing agents CW, CR, hydrogen peroxide (H_2O_2), sodium dodecyl sulfate (SDS), sodium chloride (NaCl), or sorbitol (S). None of the strains was affected by the stress-inducing reagents during either yeast-like or filamentous growth.

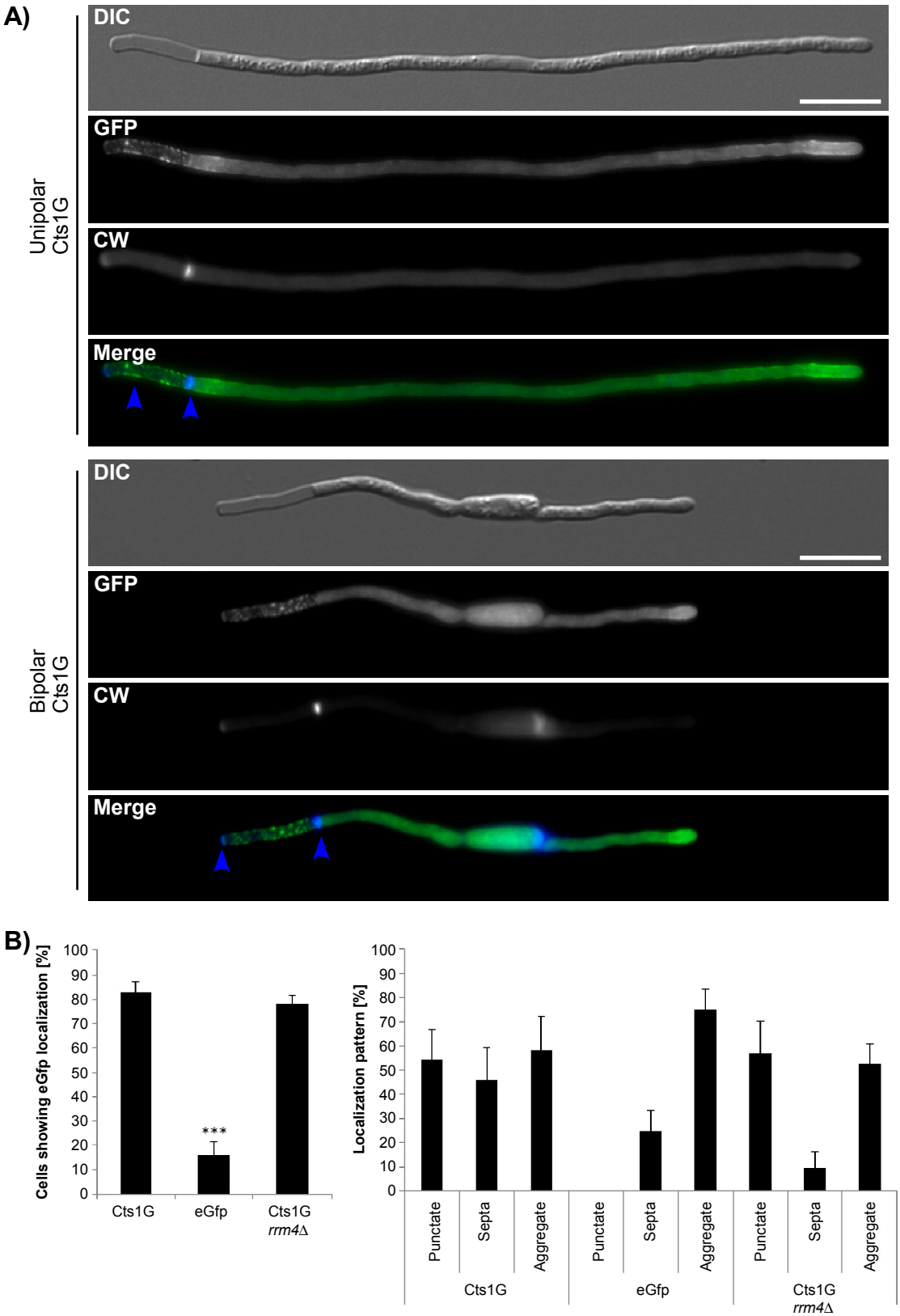


Fig. S9: Cts1G localization to empty sections is independent of Rrm4. (A) Co-localization of Cts1G and the cell wall marker CW in unipolar and bipolar AB33rrm4Δ filaments. Cts1G abundance gradually increases towards the hyphal tip as described for AB33 cells. Also similar to AB33 cells, punctate Cts1G accumulations occur between the 1st and the 2nd septum (septa indicated by arrowheads) in both unipolar and bipolar filaments. **(B)** Quantification of localization patterns extended from Fig. 5.

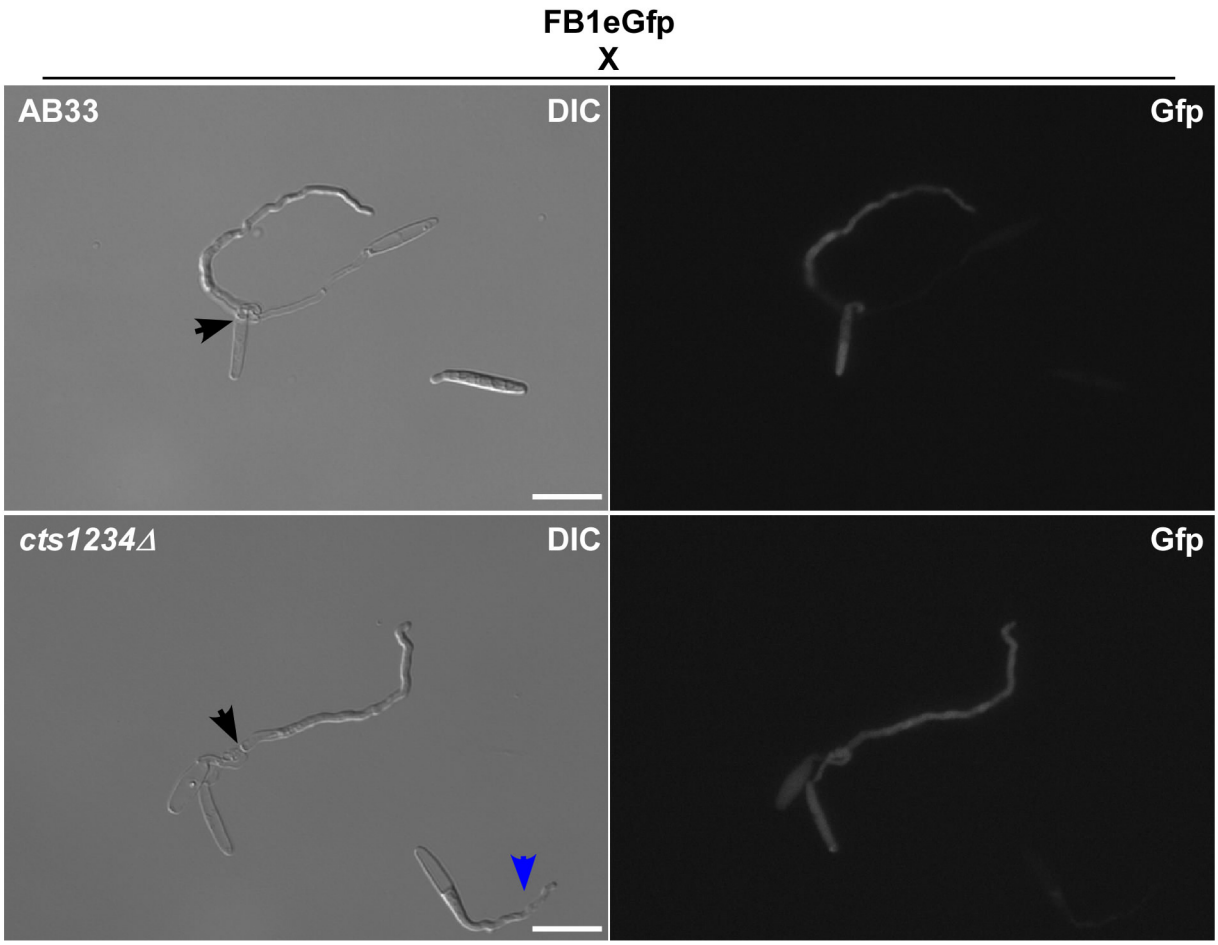


Fig. S10: Mating of chitinase deficient strains. Microscopic analysis of mating events in crossings of AB33 or corresponding chitinase deletion mutants with the compatible mating partner FB1eGfp. All chitinase deficient strains were still able to fuse with FB1eGfp. and to initialize early stages of filamentous growth (exemplarily shown for AB33cts1ΔctsΔcts3Δcts4Δ). Fusion sites are highlighted by black arrows. Hence it is sufficient if one partner provides chitinases or chitinases are dispensable for mating. Scale bars, 10 µm.