Supplementary Material

Analysis of a cellulose synthase catalytic subunit from the oomycete pathogen of crops *Phytophthora capsici*

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Table S1. Primer pairs used to assess the mycelial RNA transcript abundance of the four cellulose synthase genes from *Phytophthora capsici*.

Gene	Primer Sequence (5´-3´)	Amplicon size (bp)
PcCesA1	Forward: TATGAGATGTACGCGACGCC Reverse: GTCGGAATTGACTGGACGGT	111
PcCesA2	Forward: CGTACAATGTTTCCGCGACC Reverse: ACGCCTAGAGCGAACGTAAG	90
PcCesA3	Forward: CGGTGTCTCCCGTCTTATCG Reverse: CATGCCCTTTAGAGCAGCCT	94
PcCesA4	Forward: AAGGGGCGATTCAGTTACCG Reverse: TCGCACGGTTCAATACCCAA	107

Table S2. *Saccharomyces cerevisiae* strains used in this study.

Strain	Genotype	Source
BY4742	**α** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	ATCC, Manassa, Virginia (USA)
4015251	**α** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 fks1Δ::KanMX4*	ATCC, Manassa, Virginia (USA)
SK28[a]	**α** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 fks1Δ::KanMX4 gsy2Δ::LEU2*	This study
SK16[a]	**α** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 fks1Δ::KanMX4 gsy2Δ::LEU2 pep4Δ::LYS2*	This study
SK17[a]	**α** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 fks1Δ::KanMX4 gsy2Δ::LEU2 pep4Δ::LYS2 gsy1Δ::HIS3*	This study

[a]Derived from strain 4015251

Table S3. Primers used for the generation of mutant *Saccharomyces cerevisiae* strains.

Primer	Sequence (5'→3')	Purpose
GSY2_grcF	AACTGTGATTGAAGTTTTGACTACCTC AGAGAAAAATTTTGAATGCGTTTCGGT GATGAC	GRC amplification
GSY2_grcR	CTTTATGGTAAGATTTTTTTAATACTGT TTATATCCTCATAGGATTTACTTACGC ATCTGTGCG	GRC amplification
GSY2_seqF	TATCATGCCAGGCAGGACAG	Verification of disruption
GSY2_seqR	CAACTGCAGCTCTGGTAGCG	Verification of disruption
GSY2_seqR 2	AGGTTCCTTGGGTTTGTTGC	Verification of disruption
HIS3_seqF[a]	GCTGAAACGCAAGGATTG	Verification of genomic locus for auxotrophic marker
HIS3_seqR[a]	GCTGGGTAAGGATGATGC	Verification of genomic locus for auxotrophic marker
PEP4_grcF	TAGTATTTAATCCAAATAAAATTCAAA CAAAAACCAAAACTAACATGAGCAGA TTGTACTGAGAGTG	GRC amplification
PEP4_grcR	AGATGGCAGAAAAGGATAGGGCGGAG AAGTAAGAAAAGTTTAGCTCATTCTCC TTACGCATCTGTG	GRC amplification
PEP4_seqF	GAAGTTTGGGTAATTCGCTGCT	Verification of disruption
PEP4_seqR	GGGCTACCCGCATATAATGAC	Verification of disruption
PEP4_seqR2	GACAGGTGTTGATTGGTCTAATGG	Verification of disruption
LYS2_seqF[a]	CTTCCTGGTTGATGGTCAC	Verification of genomic locus for auxotrophic marker
LYS2_seqR[a]	TGCGAGGTTTTCTTGGTC	Verification of genomic locus for auxotrophic marker
GSY1_grcF	GCTCACCAAGCTCTTAAAACGACTTCG CTGTGATGCTGC	GRC amplification
GSY1_grcR	TTCTTTTTTTTTCTCTTGAACTCGAAAC CATCTTAACTTCTGCTAACATATCTT	GRC amplification
GSY1_seqF	AACAATGACAAGCCACAAGAC	GRC amplification and verification of disruption
GSY1_seqR	GAGCCTGCCGGCTTATAGAG	GRC amplification and verification of disruption
GSY1_seqR 2	GATCGCAATCTGAATCTTGG	Verification of disruption
LEU2_seqF[a]	GCTGAAACGCAAGGATTG	Verification of genomic locus for auxotrophic marker
LEU2_seqR[a]	GCTGGGTAAGGATGATGC	Verification of genomic locus for auxotrophic marker

[a]Primers for verification of dysfunctional genomic loci for auxotrophic marker genes were designed to bind outside the marker gene sequence used for replacement of the target genes by gene replacement cassettes (GRCs).

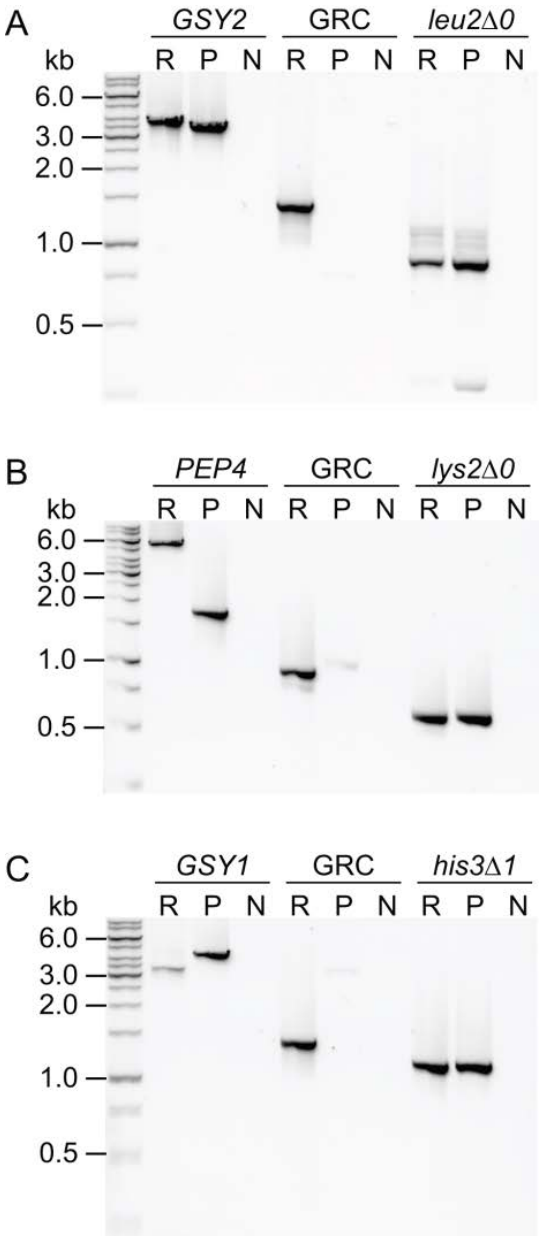

Figure S1. Construction of the yeast glucosyltransferase mutant (LoGSA).

Replacement of the target genes *GSY2* (panel A), *PEP4* (panel B), and *GSY1* (panel C) as well as integrity of dysfunctional genomic loci for the auxotrophic marker genes *leu2Δ0* (panel A), *lys2Δ0* (panel B), and *his3Δ1* (panel C) were verified by agarose gel electrophoresis of PCR products. For each gene replacement, three DNA fragments were amplified from genomic DNA of the parental strain to be transformed (P), the replacement-carrying strain (R) and a non-template control (N): a DNA fragment spanning the entire target gene locus, a gene replacement cassette (GRC)-specific DNA fragment, and a DNA fragment spanning the entire marker gene locus.

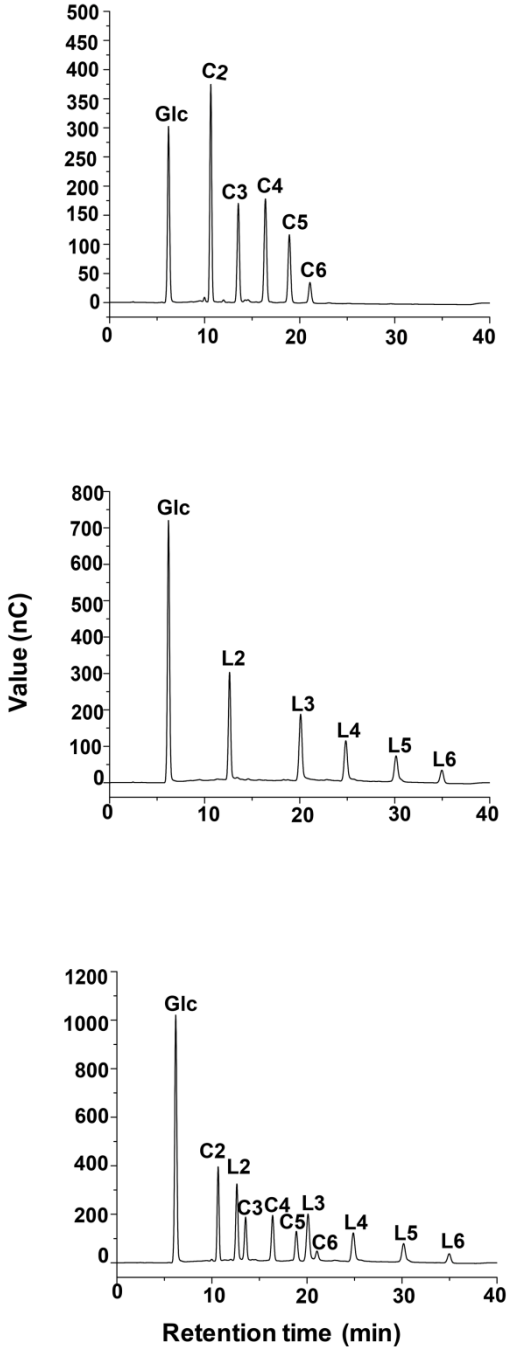

Figure S2. Chromatographic separation of standard cellooligosaccharides (β-1,4-linkages) and laminarioligosaccharides (β-1,3-linkages) by HPAEC-PAD.

Commercial standards of β-glucan oligosaccharides with β-1,4 linkages (cellobiose, cellotriose, cellotetraose, cellopentaose and cellohexaose) were analyzed by HPAEC-PAD to verify their chromatographic separation from commercial standards of β-glucan oligosaccharides with β-1,3 linkages (laminaribiose, laminaritriose, laminaritetraose, laminaripentaose and laminarihexaose). The different standards separate well, allowing the identification of the products formed by the recombinant *Pc*CesA1 by comparing retention times with these standards.

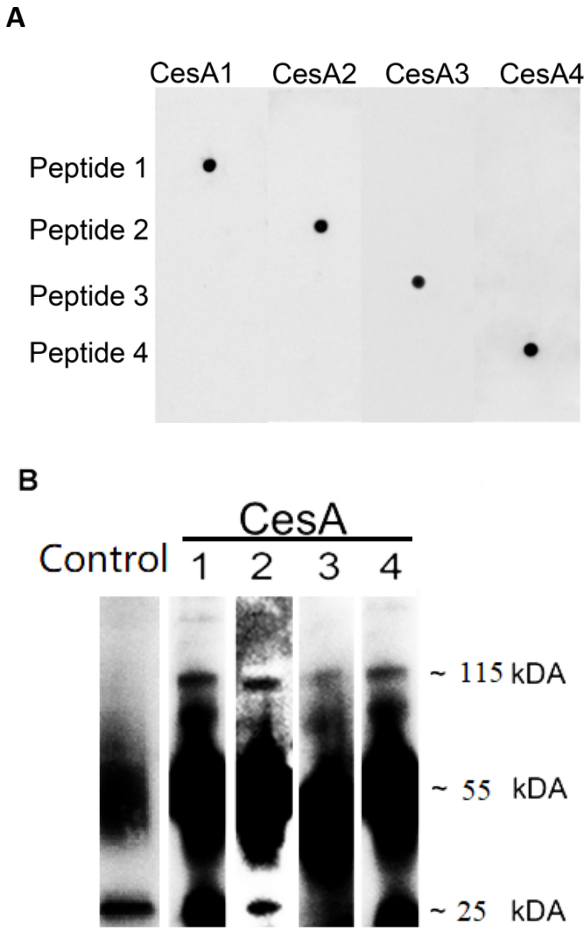

Figure S3. Dot blot analysis showing the specificity of the anti-*Pc*CesA1, anti-*Pc*CesA2, anti-*Pc*CesA3 and anti-*Pc*CesA4 antibodies (A), and immunoprecipitation analysis of the four CesA proteins from *P. capsici* (B).

(A) Unique peptides from each CesA proteins were used to raise anti-*Pc*CesA monoclonal antibodies. The specific peptides were: *Pc*CesA1-peptide 1 (SAMDIRQPGE), *Pc*CesA2-peptide 2 (NKGILETAGD), *Pc*CesA3-peptide 3(AVLSNVAFTSLQDGG) and *Pc*CesA4-peptide 4 (ANRQPPGLGA). (B) Western blot performed on the proteins immunoprecipitated by the anti-*Pc*CesA1 antibody. The different lanes were probed using the anti-*Pc*CesA1, anti-*Pc*CesA2, anti-*Pc*CesA3 and anti-*Pc*CesA4 antibodies as indicated at the top of the figure. Control: negative control performed in the absence of primary antibody. The CesA proteins detected by the antibodies have an apparent molecular mass of 115 kDa. The heavy and light chains of the mouse anti-CesA1 monoclonal antibodies detected by the secondary antibodies have a heterogeneous apparent molecular weight (due to glycosylation) centred around 55 and 25 kDa, respectively.

PcCesA1 (Cellulose synthase A1)

>PcCesA1
MFNKDQPVAV PPTDDYEMYA TPSAMDIRQP GETEIVRRSG SPDPPHRPVN SDEGGSFQDN
VITVHGYMHK QGKRAIKGPL HKSWKRRYFA LEKAKIYYFH SHLECRQYFT TRNTDLVVGA
IELKDALQLR PCARLDLPHR GFEIMTKRRV WVLCPETEEE YRLWFEGVEE AIVACGSGNI
IERKLPNVRK YYMKGITTYR FLYFLFLILS VVEIFGFVFW FVVGTQPCDS GNRTLACEDV
YDNTPDDLNC LAEPMSGWFT PPDWYLTLAG VDDVVCFHNP PIAQWVSFFA LIVAELVSMV
LGSLYYLGMW KPVRRGAHYF DEFDPKVPDD LWPKIDVLLC HYSEPAEETI DTLMACMNLQ
YPPHLLQIYV LDDGYCSTKW TKGNPVPAIE LNKVVLEKSG DLRQEVAQFM YDRVCDPNED
MEVYAWRKLH SSANLPSASR PKVVNRADCA VGSFRDDYRY PGLPHVTYVG RVKPEMHYSK
AGNINNCLYN EGANGRYMLL LDSDMQPHPK FILATLPFFF DDEDRQYKNK YSCSCTGCQN
VAKMCCASCK IAGVPEERIS YCSKECFENA MHVQSDLHRR QVNGTLSDVR ATKKELRCMN
CDSKLGKSGV CRKCNTNNNN GDTDVQILHT YSDDVRDNAV GFVQTPQYFR DCVQLQIGDP
LGHRNSTFYD AIQTGQDGYD CASFAGTNAL IRREALDSIG GIQYGTLTED CYTGERLVSM
GWKALYFRKD FEGEAEERVR LAEGLIPDSV AGAMAQRKRW AKGNFQTALM KKNKNVTDPE
WKRPHVDIPK YRKPSNFMRR VFYLNSTLYP IDSIPVILLY YITLYFLYTG YAPIFVNGLR
ILVALVPKLI VQGLLSALST RGVENNDVVR SQETRFVYAF TNFTAMLGAI VWKFTGRKSR
WLNKRDATRG SLAELPNVLV FSGAVFGIIW AMVRYIVAYY NRVYSHGDSM LCAAILMGFY
IAYNLGPSVR MSIQEYFGWS YQSLMDQGNF MGSISIAVGL MFIALWVHVE KPVEG

PcCesA2 (Cellulose synthase A2)

>PcCesA2
MYGNDKQSLM KHEDYELHGT PATGDNDGGA GFYAQEGRPM MQQGYVDPRG PALPPMNVSD
AVGLGSQRDN IISVHGYMHK QGKRTIKGPI HKSWKRRYFA LEKAKIYYFH SHLECRQYFT
TRNADLVVGA IELKDALQLR PCARLDLPHK GFEVHTKRRV WVLCPETDDE
YRMWFQGVER AIVANGAGNI IERKLPNVRK YLMKGNQTYR FFYFLFLIAG IVELLAIVFW
FVIGLEPCDA SRLEVDCETI TITSLETLRC SAQPFSGWFT PPNWYLKIAD VENVQCFRDP
PIPQWVSYFA MLFAEILTFA LGVLYYLGMW KPVRRGAHYF DEFEPPVPDE LWPKVDVLLC
HYSEPAEETI DTLMACMNLQ YPPHLLQIWV CDDGYCKAKW TKGNPVPTVE LNKGILETAG
DLRQEVAQFM YDRVCDPNED MEVYAWRKLH SSANLPSPSR VKVVNRADCA VGSFRDDYRY
PGLPHVTFIG RVKPDVHYSK AGNINNCLYN EGANGRYLII LDTDMQPHPK FILATLPFFF
DDEDRQDKAK YICCGIGCNA VAKLCCASCQ IAGVPEEQIS YCSKDCFENA MHVQSAVHRR
QVNGTMSETR QSKIDMRCMN CDSKLPKNGV CRKCGNKGAD GEDVSSLHTY SDDVKDNAVA
FVQTPQYFRD CIQLQIGDPM GHRNATFYDA IQTGQDGYDC ASFAGTNAMF RREALDSIGG
IQYGSLTEDC YTGQVLCSMG WKAQYFRKDF EGEPSERIRL AEGLIPDSVA GSLAQRKRWA
KGNFQIALMN KKTQYFDPEW KLPEAQVPSY HKSNKFMRRV FYFNSTLYPL GSITAILFYY
ITLYFLYSGY APIYMAGARL VYALVPKLFV QGVLSALSNR TVENSDVIRS QEVWFAYAFT
NCTAVLEAFW WKITGKEPKW FNTGGASRGS TAELPNVIIF FGTVVGVLWS VVRFLAGYNS
IQTSHGASLL FASLMMGLFI AVKLAPSVRM SIQEYFGWSY ESLTDQGNVV GSISIAFGLV
FITLWVWIEE PTSNPF

PcCesA4 (Cellulose synthase A4)

>PcCesA4
MANRQPPGLG ALPEDAQYSQ TPLSGVQYHE QLSSAAAPGK KLLSQSTMDV QNTINELTKA
KEHEELGKIT VHGWMHKQGS RKFKGPVAKS WRKRYFALEG AKMYYFHSDV DCRKYFNSRN
GELVVGAVDL RDAFKLEQSE RLDLPARGIV IHTRHRAWLV CPETDQDFTM WFDALEFTVM
SAGSGNVVKR DLPNVRVYEM KGRFSYRFWY VIFVITALIE LAGIVLWFPL GIEPCDVKYK
TDSCDEIQLL YADTLQCGDK PFNGVWDPPQ WYHWSAGIET VQCFKEPHIG DWVSYFLFYL
AEFISISLGF LYYLGMWKPV RRGARYLRDF EPHFPPEKWP TVDILLCHYA EPAEDTIATL
EKIMNLDYPP HLFHVWICDD GYCKSKWEAG AQVPKVSVNT GVIEEAGDVR HEVAQFMYDR

VCESYELEVD EWRKEHTTVK MPTNANPRIV NRSDCAVGSV RDDYHYHGLP KLTFVGRIKP
PVHHSKAGNI NNVLYNEGAI GRYAIILDND MKPHEMFIQA TLPFFFDAPQ NSKITRCCAP
GCGDIGKICC ALCQAAGVPE AQIMYCSKDC YNASGHTKSS VHRRQTQNTM SERMMCASCG
SKINQKKGLC RKCNRAVSQR DSNQFVGVSA DDYSDHVSVN QVGYVQTPQY FEDCLQLRLG
DPCGHRNSTF FDSAQTGMDG YDCASFAGTN AIFRREALDS VCGIQYGSLT EDAYTGKMMV
DKGWKGYYFR KDLEGEEADR IRLAEGAVPE SVAAALAQRK RWAKGNFQIF LRNKKSLVDP
EWTAPVVELP PKRKINKFMR WVFFMNLTVY PIGSFPAIFF FYITGYFLYT GQAPIYTSGL
RLLMALVPKI VAQSILSALS NRTVDNDDVL RSQQTWFSYA FVHVMAVFET IYWKITGKEA
TWANTGALGG NSPMELPNLI VFLAMIFGMM WDTVRYFAGY NNAATTHGTP LYFASLFLGG
FLASQLGPMV RMSLQTYFGW SHKSLTDQGN IVGSFSLAFV LIILCIWVYV ETPNHSIFG

Figure S4. Peptides identified by mass spectrometric analysis of the different CesA proteins that co-immunoprecipitate when using anti-*Pc*CesA1 antibodies and CHAPS extracts of mycelial microsomal membranes from *P. capsici* (see Materials and Methods (main text) for experimental details).

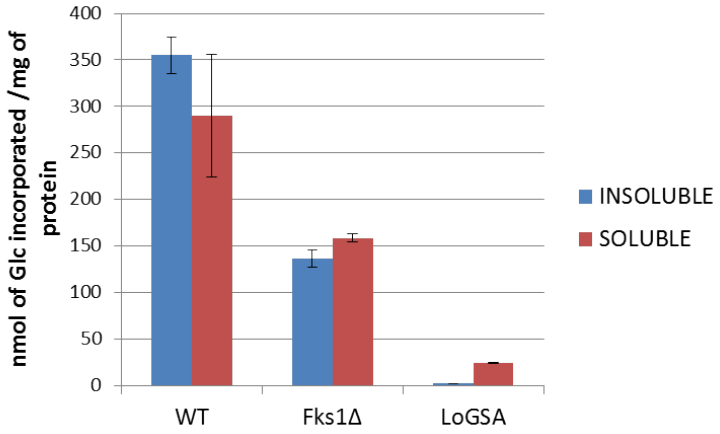

Figure S5. *In vitro* glucan synthase activity of microsomal fractions isolated from *S. cerevisiae* BY4742 (wild-type), 4015251 (Fks1Δ) and LoGSA strains. The soluble and insoluble products formed in the reaction mixtures were quantified as detailed in Materials and Methods (see main text). Data represent the means and standard deviations of a representative experiment performed in triplicate.

>PcCesA1
MFNKDQPVAV PPTDDYEMYA TPSAMDIRQP GETEIVRRSG SPDPPHRPVN SDEGGSFQDN
VITVHGYMHK QGKRAIKGPL HKSWKRRYFA LEKAKIYYFH SHLECRQYFT TRNTDLVVGA
IELKDALQLR PCARLDLPHR GFEIMTKRRV WVLCPETEEE YRLWFEGVEE AIVACGSGNI
IERKLPNVRK YYMKGITTYR FLYFLFLILS VVEIFGFVFW FVVGTQPCDS GNRTLACEDV
YDNTPDDLNC LAEPMSGWFT PPDWYLTLAG VDDVVCFHNP PIAQWVSFFA LIVAELVSMV
LGSLYYLGMW KPVRRGAHYF DEFDPKVPDD LWPKIDVLLC HYSEPAEETI DTLMACMNLQ
YPPHLLQIYV LDDGYCSTKW TKGNPVPAIE LNKVVLEKSG DLRQEVAQFM YDRVCDPNED
MEVYAWRKLH SSANLPSASR PKVVNRADCA VGSFRDDYRY PGLPHVTYVG RVKPEMHYSK
AGNINNCLYN EGANGRYMLL LDSDMQPHPK FILATLPFFF DDEDRQYKNK YSCSCTGCQN
VAKMCCASCK IAGVPEERIS YCSKECFENA MHVQSDLHRR QVNGTLSDVR ATKKELRCMN

CDSKLGKSGV CRKCNTNNNN GDTDVQILHT YSDDVRDNAV GFVQTPQYFR DCVQLQIGDP
LGHRNSTFYD AIQTGQDGYD CASFAGTNAL IRREALDSIG GIQYGTLTED CYTGERLVSM
GWKALYFRKD FEGEAEERVR LAEGLIPDSV AGAMAQRKRW AKGNFQTALM KKNKNVTDPE
WKRPHVDIPK YRKPSNFMRR VFYLNSTLYP IDSIPVILLY YITLYFLYTG YAPIFVNGLR
ILVALVPKLI VQGLLSALST RGVENNDVVR SQETRFVYAF TNFTAMLGAI VWKFTGRKSR
WLNKRDATRG SLAELPNVLV FSGAVFGIIW AMVRYIVAYY NRVYSHGDSM LCAAILMGFY
IAYNLGPSVR MSIQEYFGWS YQSLMDQGNF MGSISIAVGL MFIALWVHVE KPVEG

Figure S6. Peptides identified by mass spectrometric analysis of the recombinant *Pc*CesA1 protein purified by immobilized metal affinity chromatography (IMAC) (see Materials and Methods (main text) for experimental details).

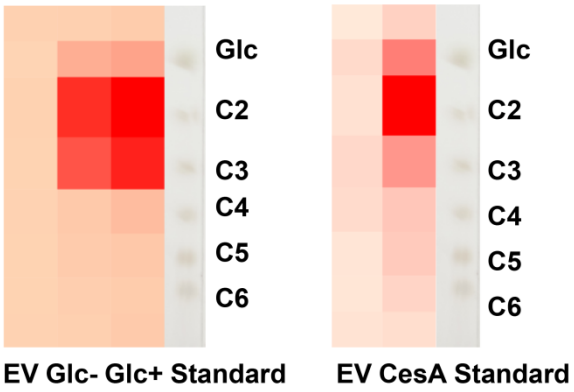

Figure S7. TLC analysis of the water-soluble products formed *in vitro* by the recombinant *Pc*CesA1protein in CHAPS-extracts of yeast microsomal membranes (left panel) or purified by IMAC (right panel).

Radiometric quantification of Glc incorporation into soluble β-glucan products was performed after separation of the oligosaccharides synthesized *in vitro* by the recombinant *Pc*CesA1 protein in the presence of radiolabelled UDP-Glc, as described in Materials and Methods (main text). Glc-, reaction performed in the absence of added glucose. Glc+, reaction performed in the presence of 250 µM glucose in the assay. The assay on the purified recombinant *Pc*CesA1 protein (CesA, right panel) was performed in the absence of added Glc. EV, control performed in the same conditions as for the CHAPS-extracted protein sample or the purified recombinant *Pc*CesA1 protein by using protein preparations from the yeast strain transformed with the empty expression vector. Standard, glucose (Glc) and cellooligosaccharides of degrees of polymerization of 2 to 6 (C2-C6). Standards were run on the TLC plate at 1.5 mg mL^{-1} and stained with H_2SO_4 in ethanol. Sample lanes were cut into squares and analyzed radiometrically to locate the reaction products. The intensity of the red colour reflects the levels of radioactivity detected in each square.