Supporting information - Figures

 subtilis Lichenzes
 licheisi fürmis Lichenzes
 PaGaić (Dessert Poplar) XP 011023441.1
 PEGAIć (Black Cothonevod) ZP 002301319.1
 SpEGIć (Purpla Pitcher) Spuritla.0024804
 MesEGIč (Caszava) cassava4.1016669
 ToEGIć (Caszava) cassava4.101669
 ToEGIć (Caszava) cassava4.101670
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 ToEGIć (Sassava) zw 074510867.1
 ShEGIć (Duras) zw 00150346.1
 ToEGIć (Zassava) zw 070134988.1
 VaEGIć (Cassava) zw 070134988.1
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 ToEGIć (Rassava) zw 07048632.1
 ToEGIć (Rassava) zw 07048632.1
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PEGGI (Desst PopLar) XP 011023441.1
SpEGI (Casseva) convocid XP 002301319.1
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	•••		TGGSFFDPFNGY TGGSFYEPFNNY	NSGFWQKADG NTGLWOKADG	YSNGNMFNCT YSNGNMFNCT	RANNVSMTSL. Rannvsmtsl.	GEMRLA Gemrls	LTSPA LTSPS	INKFDCGE INKFDCGE	NRSVQTYGY NRSVQTYGY	. Lyevr	MK.PAK MK.PAK
.1	MADP	.GINHETOPIN.		QIAID		YTPEACTHCPES	NSITLT	YDH	RGGAR	WRSTTRFLY	. TFSSL	IQCPKG
0430.1	MADP	. EIHHETQPIN.		QIAID		TPEACTHCPES	NSITLT.	YDH	RGGAR	WRSTTRFLY	. T F SSL	IOCPKG
	MADP	. TLHHETHPIK. . ADHHQTQPIK.				YTPEACTHCPVS YTPEACTHCPVS	NSITLT NSITLT	YDH FdH	RGGAR	WRSTTR F LY WRSTTR F LY	J.T F SSL J.T F SSL	IQCPQG IQCPRG
	MADP	ALHEOIOPIK.		FIAID		YT PEACTECPVS	NSITLT	FDE	RGGAR	WRSTTRFLY	.TFTSL	IQCPKG
	MADPALHPE	TPTHNQTQPLK.		OIAID		TPEACTHCPDA	NTITLT	YDH	RGGSR	WRTTTRFLY	TSSL	IQCPRG
	MADPALHPE	TPTHHQTQPLK. SLHHEAOPLK.		PIAVD		YTPEACTHCPDA YCPESCTHSPES	NTITLT Stitlt	YDH FdH	RGGAR	WRTTTRFLY WRSTTRFOY	. T F SSL . T F SSL	IQCPRG IOCPKG
	MADP	VOSLEQTEPIK.		FIAID		YTPEACTHCADS	NSITLT	FDE	RGGAR	WRSTTRFLY	.T F SSL	IQCPKG
	MADPALHPE	TPSHEKTOPLK.		QIAID		TPDACTHFTDS	NTITVT	FDH	RGGAR	WRTPTRFLY	. TFSAL	IQCPSG
		. SLHHETQPLK.		QIAVD		YTPEACDHCPAA YTPEACNHCPIS	NSIALT Ntialt	FDE	RGGAR	WRTTSRFRY WRSTTRFLY	. T F SAL . T F TAK	IQCPGG IOTPKG
	MSDP	EIQPLK.				YTPEACIHCPDT	NTITLT	YDH	RGGAR	WRTTTRFLH	. T f ttk	FQSPKG
	MADPALHPA	PLHQTQPLK.		EITID		YTPEACSHCATS	NSIALT NTITLT	YDH	RGGAR	WRTASRFRS	. T F RAH . T F SAL	IRCPGG
	MADA	LPLSHATQQLK.		QIAID		YTPEACSHCAIS	DTITLT	YDI	RGGAR	WRTPTRFES	J.FFSAL	IKCPEG
	MAAKSEVC SWEPH	PLEPDGTEPLQ.		QIAVD		YCPEACLEEREA	GEIHVT	YDH	RGGAR	WRSRRRFRP	SAVATT	IRAPAG
	MASESEACSWEPH	LLHPNG TEPLH . LLHPNG TEPLH .		RIAVD		YCPEACLHERHA YCPEACLHERHA	GEIHVT Geihvt	YDD	RGGAR	WRSCCRFLP WRSCCRFLP	SAVATT	IRAPAG Irapag
	MASESE . CVAVAEPPEVHVI	ELEPDGTEPLA.		BIAVD		YCPEACHHASED	GEIEVT	YDD	RGGAR	WRSRCRFLP	GAVAAT	IRAPAG
	MAPECDSAA	AAADDGTEPLA.		RIAVD		YTPEALEHARAA	GEIHVT.	YDH	RGGAR	WRSLRRYLP	GAVAAA	IRAPAG
	MASE	HLRPDGTEPLA. HLRPEGTEPLA.	• • • • • • • • • • • • •	RIAVD.		YTPDACRHAPES	GEIHVT Grihvt	YDD	RGGAR	WRSRSRFLP WRSRGRFLP	GAVAAA CAVAAT	VRAPAG Vrapag
	MAEV	. PSIHRTEILK.		QIAVD		YCPEVCHHSPEA	GEIHVT	YDH	RGGAR	WRS PARFRE	. T F GAR	IRCPAG
1.1	MADTPTHTEPLSLTTP	TALINGTELLD.				YCSDVCTHHTDS	NGIHIT	FDH	RGGAR	WRSKGRFRY	. TIGAR	IKCPAG
1.1	MEKV	. PDLEKTEELS.		AIAVD		YCPEVCQHFPEQ YCHEVIDHSKDA	REISVT Betats	FDE	RGGAR	WRSTRRFSS WRTTHRFES	. TFSAK	IKTPSG
	•••••••••••••••••••••••••••••••••••••••			PT		AFDHIKYFNGG	NEIQLH	LDK	YTGTG	FQSKGSYLF	. H F SMQ	MKLVPG
	•••••••••••••••••••••••••••••••••••••••	•••••••••••••••••••••••••••••••••••••••	• • • • • • • • • • • • • •	····		GPOHORVDOG.	.SLTIW	LD8	TSGSG	FKSINRYRS	J. Y <mark>F</mark> GAN	IKLQSG
				Lic	cheninase Loop)						
	β7 L8	β8	L9 β9 T-TT	L10	β10	<u>L11 β11</u> TT	L12 β	12 L13 β	13			β14
	90 100	110	120			149 15		160		170		180
	MT. CIVES FFITGPDGT MV. CIVES FFITGPDGT MTSCLIFFITGSL.COD MTSCLIFFITGSL.COD MTSCLIFFITGSL.COD MTSCLIFFITGSL.COD MTSCLIFFITGSL.COD MTSCLIFFITGSL.COD MTSCLIFFITGSL.COD MTSCLIFFITGSL.COD	PWDEIDIEFLG.	KDTTKVQFNY KDTTKVQFNY	YTNCACH	HRIVDLG	DAANAY TAF	D QPNSIN OPNSIN	WYVDGOL	CHTA	. TNQ	IPT.TP IPQ.TP	GKIMMN
	NTSCLNFNIYLCSL.EGDE	SODEIDFEFLG.	KDKTIVOTNY	YASCTON Yascton	REINDLG	TDCSDAFIE VVI	CPSFMI	WLIDGKV	REVE	.KREV E	TP. KP	MLLYAS
.1	NTSCLNFNLILSSL.EGDK	SODEID TETLG.	KDKSIVOTNF	YASCTON	RETHDLG	DAANAII TAF DAANSYITAF TOCSDAFIEVVI TOCSDAFIEVVI TOCSDAFIEVVI		WLIDGEL	REVE	.KREG	FPE KP	MFLYAS
	NTSCLNFNIYLSSL.EGDK	SODEIDFEFLG.	KDKTIVOTNY KDKTIVOSNY	TTTCTCN YTTCTCN	R.QIHDLG				/RKEE	RKDGV	TPE KP	MPLYAS
	NTSCLNFNIYLSSL.EGDK	SODEIDFEFLG.	KDKTIVOTNY	YTTCTCA	REGINDLG	DCSDGF	K N PD SII	WVIDGKV	REAE	KKEG	VPE . KP	YSG
	ITSCLAFAITLSSL.EGDK	SODEIDTETLG	KDRTIVOTNY	YTT <mark>C</mark> TCN YIGCTCN	KEFHDLG	DCCCCCC	K GQDLII	WIT WID CHILL	RRVE	RKKG	TPH. KP	MYLYAS
	ITSGLNFNIYLSSL.EGDK NTSGLNFNIYLSSL.EGDK NTSGLNFNIYLSSL.EGDK	SODEIDFEFLG	KDRTIVOTNY	YTGCTCN YTACTCN	KEEFHDLG REAIHDLG	DCSDGFEL VI DCSDGFEL VI	K GPDLIC	WLTDGKL	RSVV	. RKKG E	AFPH.KP SFPQ.KP	MYLYAS
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	NTSCLNFNIYLSSL.EGDK	SOBIDFEFLG.	. RDMTIVOTNY	YTTCTCN YTECACN	REEIHDLG	NCSEGF	K GPDLIČ K SKDVIJ	WFINGEM	/RRAE	REEG	PDN ED	MYLYSS
	NTSCLNFNLYLSSL EGDK	SODEIDFEFLG	KDKTIVOTNY	YTEGAGN YTTGTGN YSNGNGN		DCSDGFIEVVI DSSDGFIEVGI	K DKEYII	WVIDGKL WWIDGKL	RRVE	. RKEG E . RKEG D	FPD.KP	MFLYAS
	NTSCLNFNFYLSSL.EGDK	SODEIDTEFLG.	KDTKIVOTNY		RUKVHELG	DSSDGF	K <mark></mark> G P F G I I	WLVDGEV	REEG	GGDG	TPE.KA	MFLYAS
	DTSCLNFNIYLSSL.EGDK	SODEIDFEFLG.	RDRTIVOTOF	YTSCTCN YSECACN	REQIHNLG	DASDGF	K GPDLII V GSDAIR	WRVDGKV	/RREE	. RREG E	TPE.KA	MFLYAS MFLYAS
	DINCLNFNLYLSSO . EGDE	DEIDFEFLG.	RDRTLVOTNF	FSGCACN	NRIHHLG	DASEGFERAI	A GROTIE	WRVDGEV	/RREE	RKEG E	STPE.KA	MFLYAS
	DTSGLNYNIYLSSL. EGSK	DODEIDFEFLG.	HOKRAVOTNY	HVNCDCG	REQIENLY	DSSDGFERAI	A DAATIE	WRVDGEL	RREE	9926 2		MYT.YA C
	DTSCLNYNIYLSSL.EGSA	DODEIDFEFLG.	HDKRAVOTNY	YVG <mark>C</mark> NCG YVGCNCG	REQIHALP	DSSDGFEHYAI	A DAAAII A DAAAII	WRVDGEL	/RRDE	.RREGE	NPE KP	MYLYAS
	DTACLNYNLYLSSL.EGSR	DEIDFEFLG.	HDKCAVOTNF	HVA GGG	ROIHVLP	DSSDGFHAI	A GADAII	WRIDGEL		.RVAGE	NPE.KP	MFLYAS
	DASCLNYNLYLSSL.EGCR DTACLNYNLYLSSL.EGSA	DMDEIDFEFLG.	. HDKRALOTEY	HVGCGCG HVSCACG	REMLERLP	DASDGFERMAI	A DAKATE A GAEATE	WRVDSEV	TRREE	. RREGE . RGPGPG	NPE.KP	MRLYAS MFLYAS
	DTTGLNYNLYLSSL.EGSG	DMDEIDFEFLG.	NDKRAVOTNF	TVACSCG	REAVEELP	DSSDGFUNAV	A DAEAIE A saeave	WRVDGEV		RRDGE	PMPE.KP	MFLYAS
		SODEIDFEFIG.	KDKTSVOTNF	YTTCACC YTTCACA	RIIHPLG	DASEDFELI	R 🗌 E PDR I I	WLVDGAV	R.EEV	.RKDGE	PWPE KP	MFLYAS
	NISCHAINITISST. ROOM				RIIHPLG	DASEDFEELI		WLVDGAV	R.EEV	. RKDG E		MFLYAS
1.1	NTSCLNFNIYLSSL.EGDK NTSCLNFNIYLSSL.EGDK	SODEIDFEFLG.	KDKTSVOTOF KDKRIVOTOF	YVDGVGG	R RIHDIG	DCSDGF	K GPDELE	WVIDGVV	TRREE	REKS	FPO. KP	MFLYAS
1.1 7906.1	NTSCLNFNIYLSSL.EGDK NTSCLNFNIYLSSL.EGDK NTSCLNFNIYLSSL.EGDK NTSCLNSSFYLSTL.EGPR	SODEIDFEFLG Sodeidfeflg Dodeidfeflg	. KDKTSVOTNF . KDKRIVOTNF . KDKCTVOTNF	YVDCVCG YTHCTCG	R.RIHDIG	IDCSDGFIE <mark>v</mark> ki IDSSQEFIEVTI	K GPDELE K EPDRIE	WVIDGVV	REE	. REKSD Lkelede	STPD. RP	CFLYAS
21.1 57906.1 1.1	NTSCLNFNIYLSSL EGDK NTSCLNFNIYLSSL EGDK NTSCLNSSFYLSTL EGDK NTSCLNSFYLSTL EGDK NTSCLNSFYLSSL EGDK DSACTVTAFYLSSQNS	SODEIDTEFIG Sodeidtefig Dodeidtefig Todeidtefig Endeidtefig Endeidtefig	KDKRIVOINF KDKRIVOINF KDKGIVOINF KDKIIIOINV RIGQPYILOINV	YVDGVCG YTHGTCG YTHGVCN	RERIEDIG REVIEDIG REVIEDIG	DCSDGFHEYRI DSSQEFHEYTI DSSETFHEYAI	K GPDELE K EPDRIE H TPTEIL	WVIDGVV WLVDGKS	RREE	.REKSD LKELEDE .RKPND .KNCKDLGV	GFPQ.KP GFPT.RP AYPM.KP KFPFNQP	CFLYAS MFLYAS MKIYSS
1.1 7906.1 1.1		SCDEIDTEFIC SODEIDTEFIC ODEIDTEFIC TODEIDTEFIC HDEIDTEFIC HDEIDTEFIC KHDEIDIEFIC	KDKTSVOTEF KDKRIVOTEF KDKGTVOTEF KDKGTIIOTEV RTGQFYILOTEV IPGKPYTLOTEV	YVDGVCG YTHGTCG YTHGVCN	R.RIHDIG R.VIHDLG R.ZIHQLG R.QRIYLW IGR.MRIHLW	IDCSDGFIE <mark>v</mark> ki IDSSQEFIEVTI	K GPDELE K EPDRIE H TPTEIL	WVIDGVV WLVDGKS	RREE	. REKSD LKEEEDE .RKPND	GFPQ.KP GFPT.RP AYPM.KP KFPFNQP	CFLYAS MFLYAS MKIYSS

	L15	β15 L16 β16	L17	
	190	200 210	-	
B. subtilis Lichenase	LWNGT. GVDEWLGS		TKK	
B. licheniformis Lichenase	LUNGA. GVDE NLGS			
PeEG16_(Desert_Poplar)_XP_011023441.1	INDAS, YICDAT NTGP	MGCDAPYVCLYKDICV	PVGTAVECSCDS	
DepC16 (Black Contract) VD 002201210 1	IWDAS.YICDATWTGP	MGCDAPYVCLYKDICV		
SpEG16 (Purple Pitcher) SapurV1A.0024s0430.1	INDAS.SIGEATWTGP	IGCDAPYVCLYKDICV		
MesEG16 (Cassava) cassava4.1 016069	IWDAS.CIDEGRWSGT	VGCDEPYVCLYKDIHV	PLGTAVECSSES	
SpEGI6 (Purple_Pitcher) SapurVlA.0024s0430.1 MesEGI6 (Cassava) cassava4.1 016069 TcEGI6 (Cacao_Tree) XP_007051935.1	VWDAS.YIAEAKWTGP	IGCDAPYVCLYKDIQV	PVATTVECSSDS	
GrEG16 (Cotton) XP 012489857.1	ACLHC.CRVFL	• • • • • • • • • • • • • • • • • •	<mark>.</mark>	
GaEG16 (Tree_Cotton)_KEG22293.1	VWDAS.YIAEGQWTGP	IGCDVPYV <mark>C</mark> LYKDIQV		
PmEG16 (Prunus_mume) XP_008232877.1	VWDAS.YIEEGRWTGK	IGCDAPYV <mark>C</mark> LYKDIHV		
PpeEG16_(Peach)_XP_007219843.1	VWDAS.YIEEGRWTGK	IGCDAPYVCLYKDIHV		
VvEG16_(Grape_Vine)_XP_002273975.1	VWDAS.YIDEGRWTGP	VGCDAPYICLYKNVNV	PVGTAVE	
CsEG16 (Orange) orangeI.1g028023m	VWDAS.HIDKARWCGP	VGCDAPYVCLYKDIHV	PVATAVECPCDS	
CcEG16 (Clementine) XP 006445161.1	VWDAS.HIDKARWCGP VWDAS.YIDKGRWTGK	VGCDAPYVCLYKDIHV		
FvEG16 (Strawberry) XP 004306845.1 EqEG16 (Rose Gum) XP 010053649.1		VGCDVPYVCVYKDIHV	PLETAVED	
		VGCDAPYVCLYKDIHV	PVRTALECSSDS	
MtEG16 (Barrel Clover) XP 013444946.1 CaEG16 (Chickpea) XP 004510867.1	IWDAS.CIAEGEWTGK	CGADVPIVCHIKDIHV	CONSTR	
MnEG16 (Mulberry) XP 010092731.1	VWDAS, YIDEGR WTGK		FORTAIL	• • • • • • • • • • • • • • • • • • • •
GnaEG16 (Soy) glyma07g08550.1	VWDAS. WVAEGEVV. WGST	GVMSLMFVSRGHSC.		
PvEG16 (String Bean) XP 007134988.1	TWNAS GISDGE WCCK	CCADERVYCVYEDTRY		
VaEG16 (Adzuki Bean) KOM57375.1	INDAS. GIAGGE NCGE	LELTSLMFVFIRTFMF HGRDAPYVCSYKDVMV	LLALLINTSELF	FLL
SbEG16 (Durra) XP 002468638.1	VWDAS, YIADGK WTGT	HCRDAPYVCSYKDVMV		NODADAADAPAVDPAADADAAAAAAAAAAAGKEKD
ZnEG16 (Zea nicaraguensis) AIZ06014.1	VWDAS, HIADGA WTGT	EGRDAPYVCSYKDVRV		APAGDPVAADAAAAAEEEKDAGAGEV
ZmEG16 (Maize) NP 001150346.1	VWDAS, HIADGA WTGT	HGRDAPYVCSYKDVRV	PTAEHSVEDAAH	APAGDPVAADAAAAAEEEKDAGAGEV
OsEG16 (Rice) NP 001048767.1	VWDAS.HINDGKWTGT	H <mark>grdapyvc</mark> syrdirv	PLALSLEDEEDP	YKCACVGDASAAIAAADAAEQVDAGDAPAAAAAA
SiEG16 (Foxtail Millet) XP 004985971.1 BdEG16 (Purple False Brome) KQK23978.1	VWDAS.DIDEGRWTGT	HGSDAPYVCSYKDVVV	PPVEFWVEDDEQC	QDADARDAPAAVVEEETDAVÄIAPAMNDE
BdEG16 (Purple False Brome) KQK23978.1	VWDAS.GVDEGRWTGR	HGRDAPYICSYRDVRV	PVSLSVEEEDAE	EEEGRDHANAGDAPDATATTCSAAVVAAAGADEE
HvEG16 (Barley) AK356496	FWDAS.GVDEGRWTGT	HGRDAPYVCSYRDVRV	PVALSTEEEEE	CODDADAGDEADAAGAAEEEEEEMDAGDGED
TaEG16 (Wheat) ACS92630.1	LWDAS.DVDEGRWTGT	HGRDAPYVF SYRDVRV	PAVALSAGEEEE	WQDDTNAGDEADAAATTCED
PaEG16 (Date_Palm) XP_008803266.1	VWDAS.YIDEGRWTGR	IGCDAPYVCSYKDVMV		κ
EguEG16 (African_Oil_Palm)_XP_010919221.1	VWDAS.YIDGGRWTGQ	IGCDAPYVCSYKDVRV	PIESIVVEEEES	к
AtEG16_(Amborella_trichopoda)_XP_006857906.1	VWDAS.YIDEARWTGA	IGCDAPYLCVYRDVKV		GPGS
SmEG16 (S. moellendorffii) XP 002960511.1	VWNAG.EVNEGLWAGP	IGCDVPYVCVYKDVFV		
PpEG16-3_(Ppatens)_XP_001783128.1	VWNAG.WVNNGEWAGC	VGCDEPYVCTYKDVQV		
Ptt-XET16A Tm-NKG1	LWNADDWATRGGLEKTDWS VWDASSWATENGKYKADYR			• • • • • • • • • • • • • • • • • • • •
THERAGI	VWDASSWATENGKIKADIK	QFIVGKIEDFKLGSCT	• <mark>.</mark>	• • • • • • • • • • • • • • • • • • • •

Figure S1: Protein sequence alignment of EG16s with Licheninases and *XTH* gene products. EG16s are named according to the first letters of their genus and species names. For each EG16, the common name (where available) for the plant of origin is given along with the accession code for the gene product. Regions of high similarity are shown in red. Secondary structure features, defined based on the crystal structure of $VvEG16(\Delta V152,E89A)$ in complex with cellooligosaccharides, are displayed and named above the alignment. Conserved cysteines among monocot or dicot EG16s are highlighted in yellow. The licheninases loop, XEH loop and catalytic strand are labelled below the sequences, stars mark the catalytic nucleophile, general acid/base and "helper" aspartic acid.

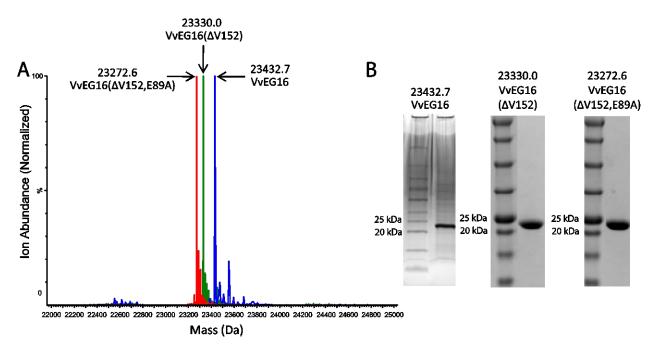


Figure S2: Analysis of purified VvEG16 proteins. A) Reconstructed intact mass spectra of VvEG16, VvEG16(Δ V152) and VvEG16(Δ V152,E89A). The purified proteins had masses of 23432.7 (expected 23432.1), 23330.0 Da (expected 23331.0), and 23272.6 Da (expected 23272.9) respectively, verifying that the protein sequence was correct, demonstrating that no post-translational modifications were present after TEV protease cleavage of the His₆ affinity tag and suggesting that all cysteine residues were reduced. B) SDS-PAGE analysis of purified VvEG16, VvEG16(Δ V152) and VvEG16 (Δ V152,E89A) after TEV protease cleavage.

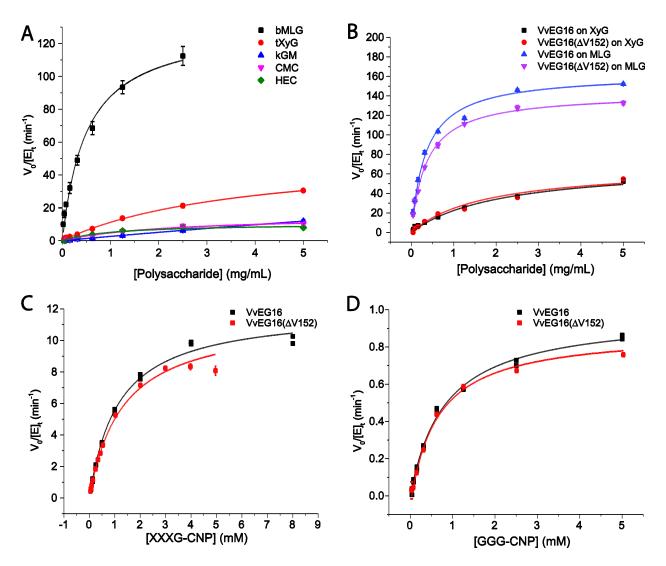


Figure S3: Comparison of wild-type VvEG16 and VvEG16 (Δ V152) activities. A) Overlay of wild-type VvEG16 and VvEG16 (Δ V152) acting bMLG and tXyG. B) Kinetics of VvEG16(Δ V152) acting on various polysaccharide substrates. C) Comparison of wild-type VvEG16 and VvEG16(Δ V152) acting GGG-CNP. Each data point represents the average of two replicates. Activity of VvEG16(Δ V152) on a variety of polysaccharides at a variety of concentrations. Kinetics were modelled to extract apparent k_{cat} and K_{M} values based on the Michaelis-Menten curves shown. Error bars represent the standard deviation of three technical replicates.

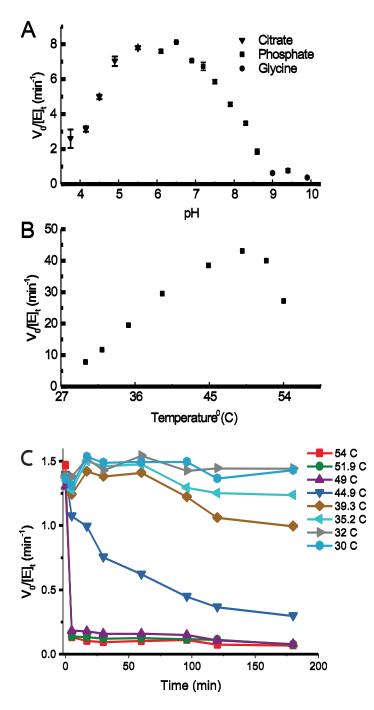


Figure S4: Dependence of VvEG16(Δ V152) activity on pH and temperature. A) Activity of VvEG16(Δ V152) on 1 mg/mL tXyG in buffers at various pH values measured using the BCA assay. Error bars represent that standard deviation of three technical replicates. B) Activity of VvEG16(Δ V152) on 1 mg/mL tXyG in pH 6.0 sodium citrate buffer at various temperatures measured using the BCA assay. Each data point represents the average of two technical replicates. Reactions were incubated for 15 minutes with no reducing agent present prior to quenching by the addition of BCA reagent. C) Thermal stability of VvEG16(Δ V152) in dithiothreitol-containing buffer. Activity on 1 mM XXXG-CNP was measured at room temperature at regular intervals during incubation of the enzyme at various temperatures in pH 5.5 sodium citrate.

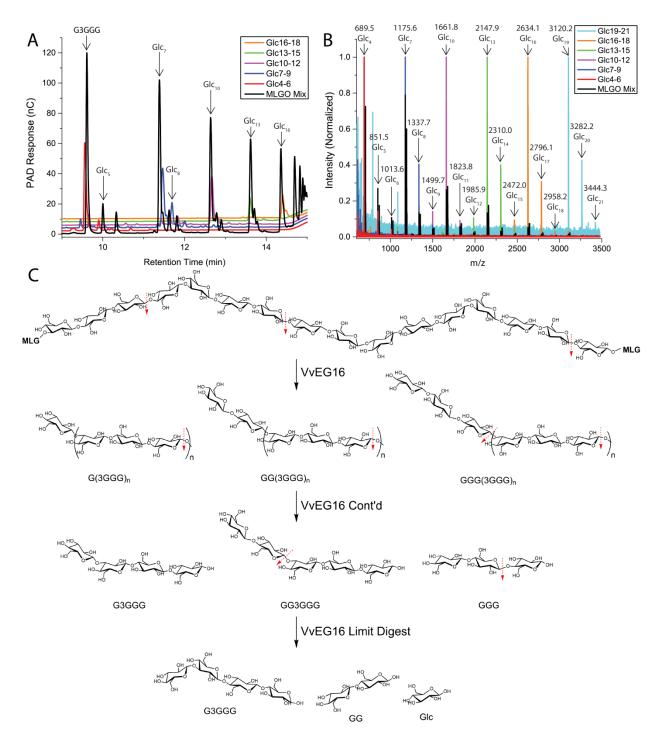


Figure S5: Identification of oligosaccharide series produced by action of VvEG16(Δ V152) on bMLG. Oligosaccharides were separated by size using a SEC column and analyzed by HPAEC-PAD (panel A) and MALDI-TOF (panel B) to determine the mass corresponding to each HPAEC-PAD peak. Panel C depicts a schematic of bMLG breakdown by VvEG16. bMLG polysaccharide (top) is initially cleaved by VvEG16 at β 1,4 bonds following cellotriose motifs. The resulting oligosaccharides (second from top) contain repeating β 1,3-linked cellotriose motifs terminated at the non-reducing end with primarily β 1,3-linked glucose (~90%) and less commonly β 1,3-linked cellobiose (~5%) or cellotriose motifs (~3%). This

gives rise to the three nested series of peaks observed in panel A. Continued digestion of these oligosaccharides gives the three oligosaccharides shown (second from bottom). Finally, slow hydrolysis of the pentasaccharide and cellotriose give the final products observed in the limit digest (figure 2C,D): glucose, cellobiose and G3GGG which cannot be further broken down by VvEG16.

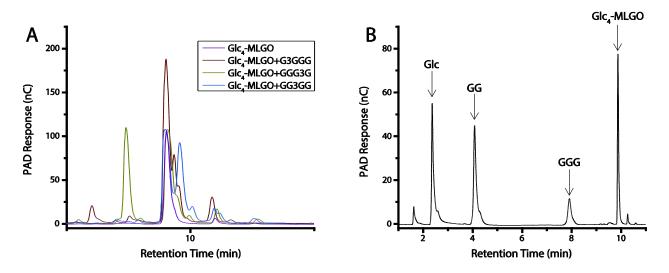


Figure S6: Identification of the MLGO produced by the action of VvEG16(Δ V152) on bMLG (Glc₄-MLGO) by HPAEC-PAD. A) 10 μ M Glc₄-MLGO was mixed 1:1 with water or 10 μ M standards of G3GGG, GG3GG and GGG3G and run with gradient B. Notably, the Glc₄-MLGO peak co-eluted with G3GGG and grew when G3GGG was added. B) Further confirmation of the identity of Glc₄-MLGO was obtained by partial digestion with an exo-acting β -glucosidase. Only G, GG and GGG were observed as products, thus confirming that the $\beta(1,3)$ linkage was found between the two non-reducing-terminal glucose residues.

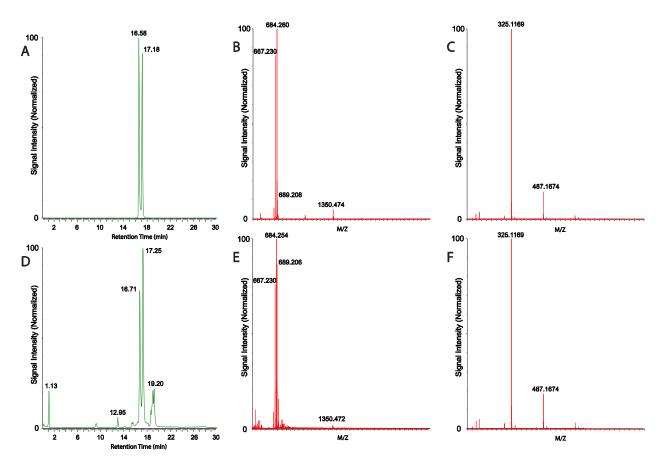


Figure S7: Identification of the MLGO produced by the action of VvEG16(Δ V152) on bMLG (Glc₄-MLGO) by LC-MS. A-C) Extracted ion chromatogram (m/z = 684.0-684.5), MS and CID MSMS spectra of The Glc₄-MLGO peak eluting at 17.2 minutes, respectively. D-F) Extracted ion chromatogram (m/z = 684.0-684.5), MS and CID MS/MS spectra of commercial G3GGG, respectively.

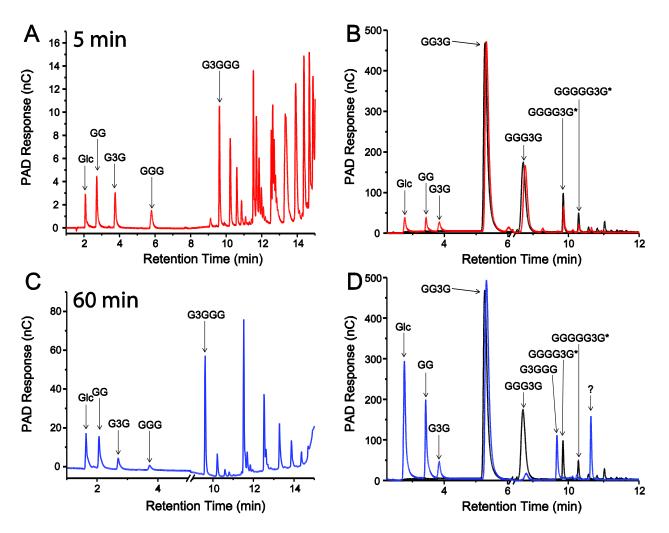


Figure S8: Time-dependent MLG hydrolysis by VvEG16(Δ V152). A) HPAEC-PAD chromatogram of the early products of MLG hydrolysis by VvEG16. B) HPAEC-PAD chromatogram of the licheninase digestion of the MLG shown in A). Licheninase-digested native MLG is shown in black and licheninase-digested VvEG16-digested MLG is shown in red. C) HPAEC-PAD chromatogram of the gel-forming MLG produced by VvEG16 digestion. D) HPAEC-PAD chromatogram of the licheninase digestion of the MLG showed in C). Licheninase-digested native MLG is shown in black and licheninase-digested VvEG16-digested MLG is shown in black and licheninase-digested NLG showed in C). Licheninase-digested native MLG is shown in black and licheninase-digested VvEG16-digested MLG is shown in blue.

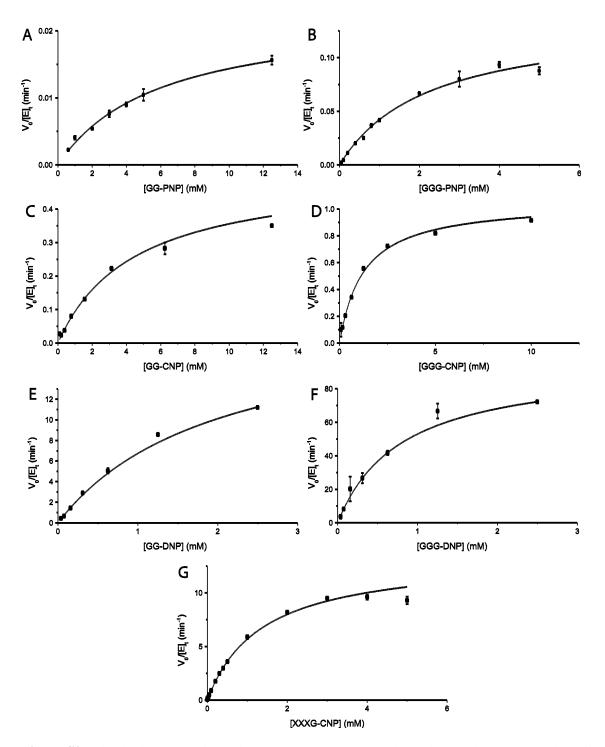


Figure S9: Kinetic data and Michaelis-Menten curves used to determine the k_{cat} and K_M values for the hydrolysis of various chromogenic substrates by VvEG16(Δ V152). Error bars are shown as the standard deviation of three technical replicates.

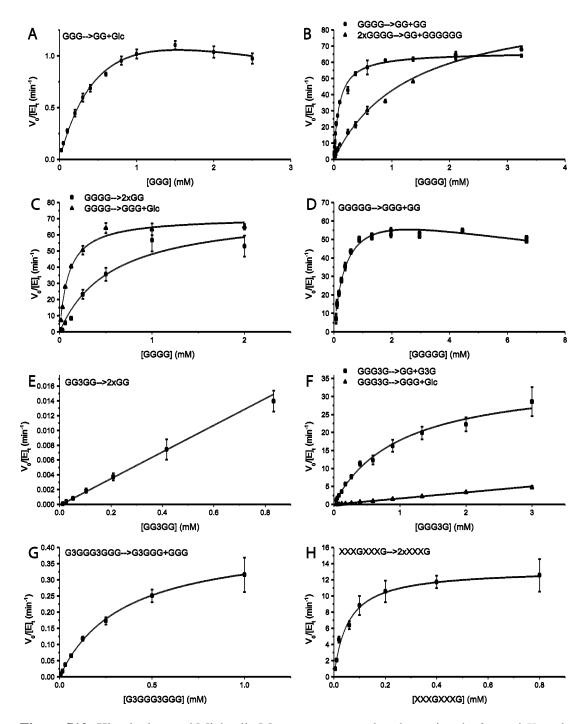


Figure S10: Kinetic data and Michaelis-Menten curves used to determine the k_{cat} and K_M values for the hydrolysis of various oligosaccharide substrates by VvEG16(Δ V152). Error bars are shown as the standard deviation of three technical replicates.

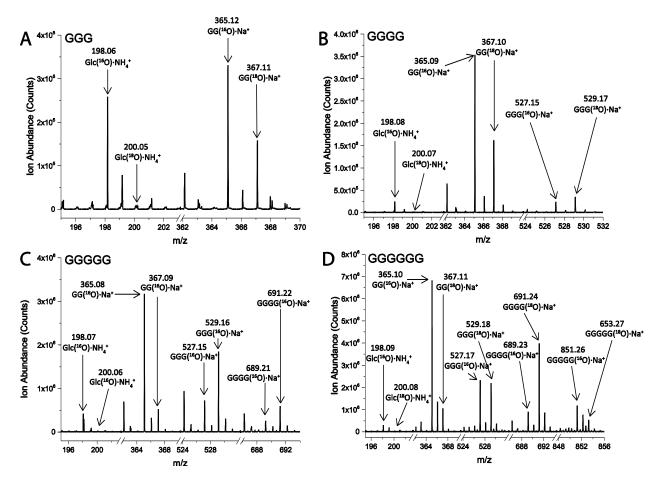


Figure S11: Mass spectra of products produced by the hydrolysis of cello-oligosaccharides by VvEG16(Δ V152) in H₂¹⁸O. A) cellotriose, B) cellotetraose, C) cellopentaose, D) cellohexaose. Peak integration data can be found in table S3.

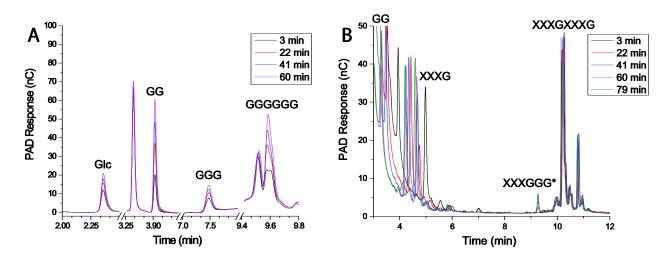


Figure S12: Homo- and hetero-transglycosylation of oligosaccharides by VvEG16(Δ V152). A) The HPAEC-PAD chromatogram of the action of VvEG16(Δ V152) on 1 mM cellotetraose over time. The increase of the peak at 9.6 minutes is indicative of the formation of cellohexaose. B) The HPAEC-PAD chromatogram of VvEG16(Δ V152) acting on 100 μ M XXXGXXXG in the presence of 5 mM cellobiose over time. A small peak is observed forming at a retention time of 9.3 minutes and, later, being hydrolyzed. Though not isolated, this peak is putatively assigned as XXXGGG.

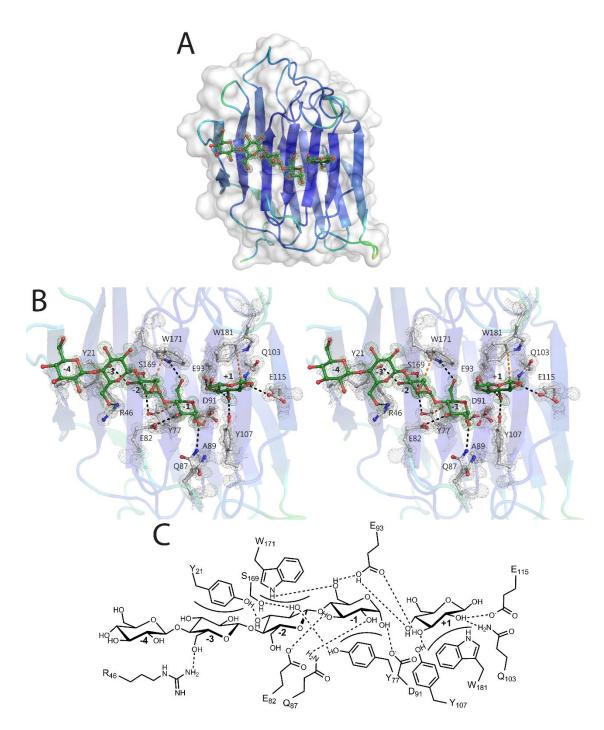


Figure S13: Structure of VvEG16(Δ V152,E89A) in complex with cellooligosaccharides. A) The asymmetric unit of this complex contains one protein molecule, shown as both a white surface representation and a cartoon representation coloured according to B-factors. The protein molecule bound to a molecule modelled as GGGG in the negative subsites and glucose in the positive subsites (both shown in green). B) and C) show the interactions between VvEG16(Δ V152,E89A) and cellooligosaccharides as in figure 3 B) and C).

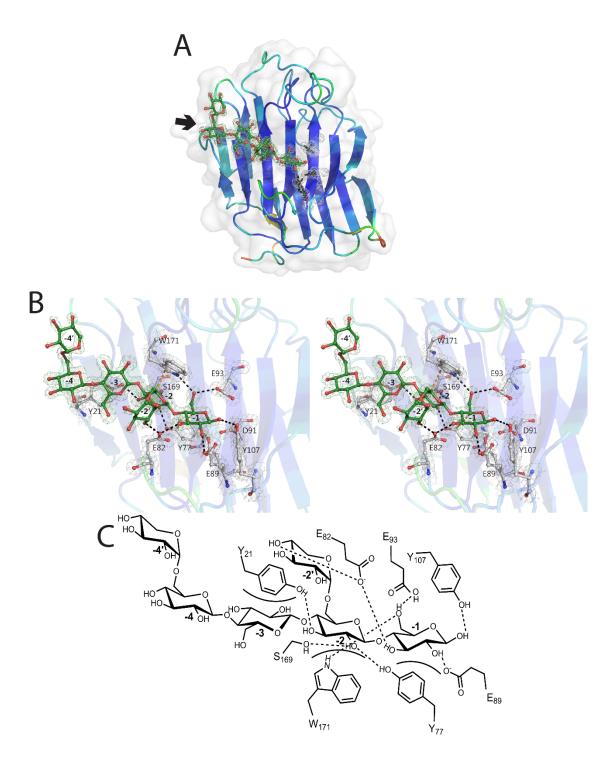


Figure S14: Structure of VvEG16(Δ V152,C22S,C188S) in complex with a xyloglucan oligosaccharide. A) The asymmetric unit of this complex contains one protein molecule, shown as both a white surface representation and a cartoon representation coloured according to B-factors. The protein molecule bound to a molecule modelled as XGXG in the negative subsites (shown in green). The location of the C22S mutation is noted with an arrow; the C188S mutation is located on the back of the enzyme from this perspective. B) and C) show the interactions between VvEG16(Δ V152, C22S,C188S) and XGXG as in figure 5 B) and C).

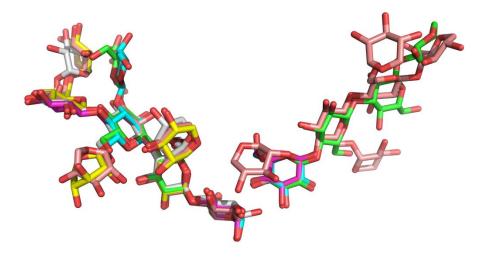


Figure S15: Superimposition of the carbohydrate ligands bound within the active site of each experimentally determined structure of VvEG16. Carbohydrates from all six chains (PDB IDs 5DZE (Magenta), 5DZF-A (Green), 5DZF-B (Cyan), 5DZG-A (Yellow), 5DZF-B (Salmon), and 5SV8 (White)) are shown.