



Supplementary Information for

Constructing a yeast to express the largest cellulosome complex on the cell surface

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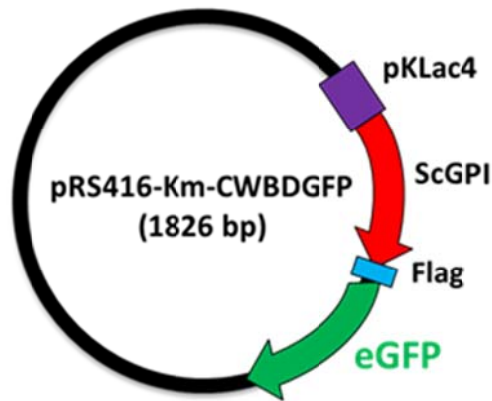


Fig. S1. Cell surface display of the *Saccharomyces cerevisiae* GPI anchor on *K. marxianus* cell wall. Plasmid constructs used for the expression of ScGPI fused with GFP.

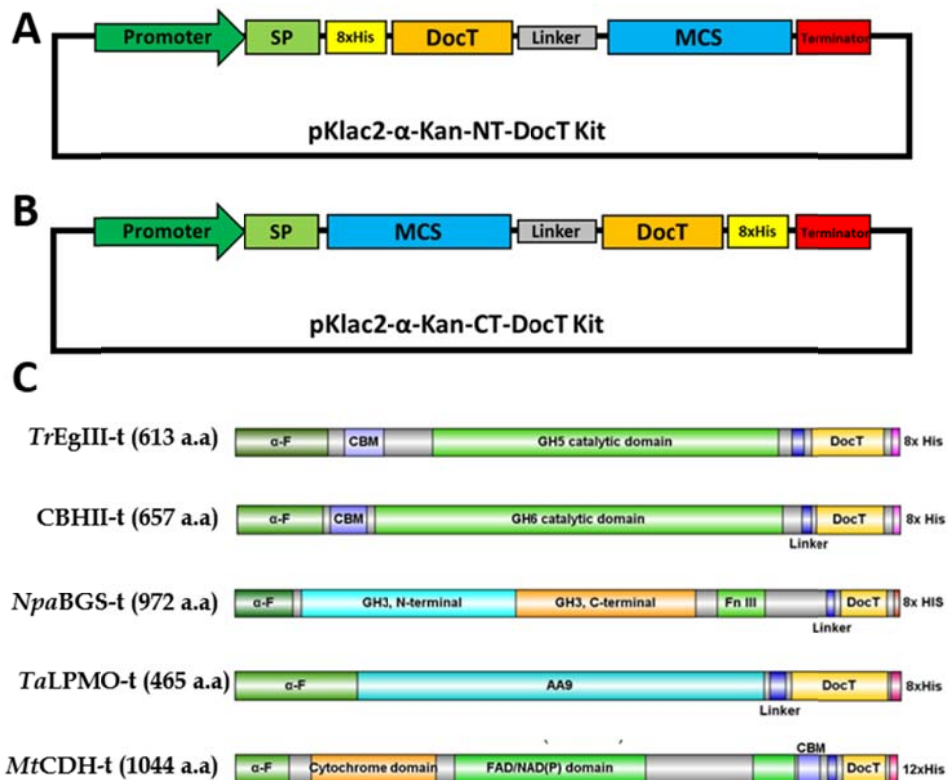


Fig. S2. Conversion of free cellulases into the cellulosomal mode. **a**, N-terminal dockerin fusion plasmid. **b**, C-terminal dockerin fusion plasmid. **c**, Domain organization of cellulase enzymes. Promoter: Lac4, SP: Signal peptide (*Kluyveromyces lactis* α -matting factor), DocT: Dockerin T from *Clostridium thermocellum*, MCS: multiple cloning site, Terminator: Lac4, α -F: α -matting factor, CBM: cellulose binding module.

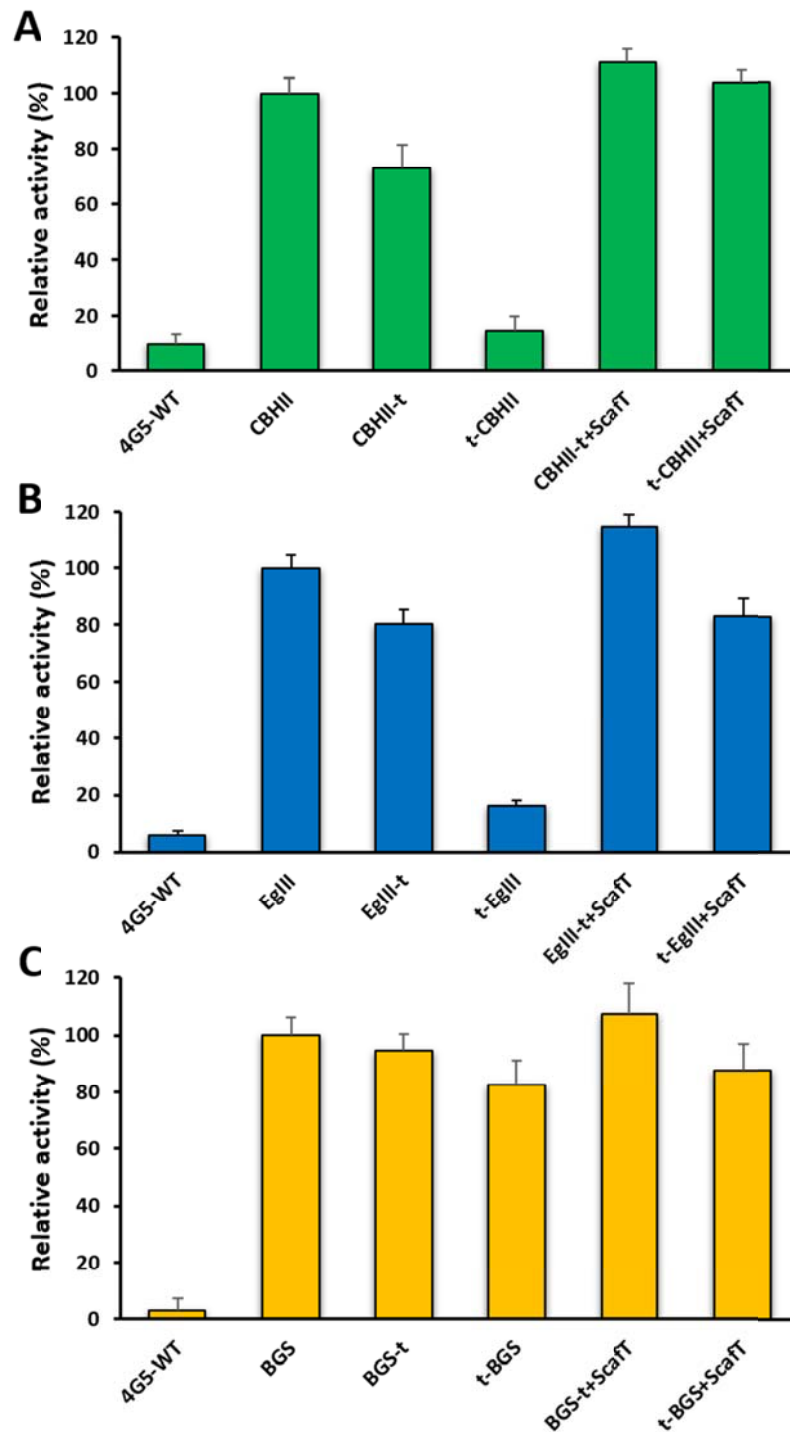


Fig. S3. Effects of dockerin fusion on the cellulase enzyme activity. The enzyme activity was assayed after the N- or C- terminal dockerin fusion. The ‘t’ represents Dockerin T from *C. thermocellum* and the position of ‘t’ denotes N- or C- terminal DocT fusion. The CBM restoration was performed by the addition of ScaFT (containing a CBM and CohT) with dockerin CBHII-t (a), EgIII-t (b) and *Npa*BGS-t (c). The enzyme activity was assayed using specific substrates, and for the CBM restoration, enzyme activity was assayed after the complex formation at 37°C for 3 h. The results were expressed as mean (n=3) ± standard deviation (SD).

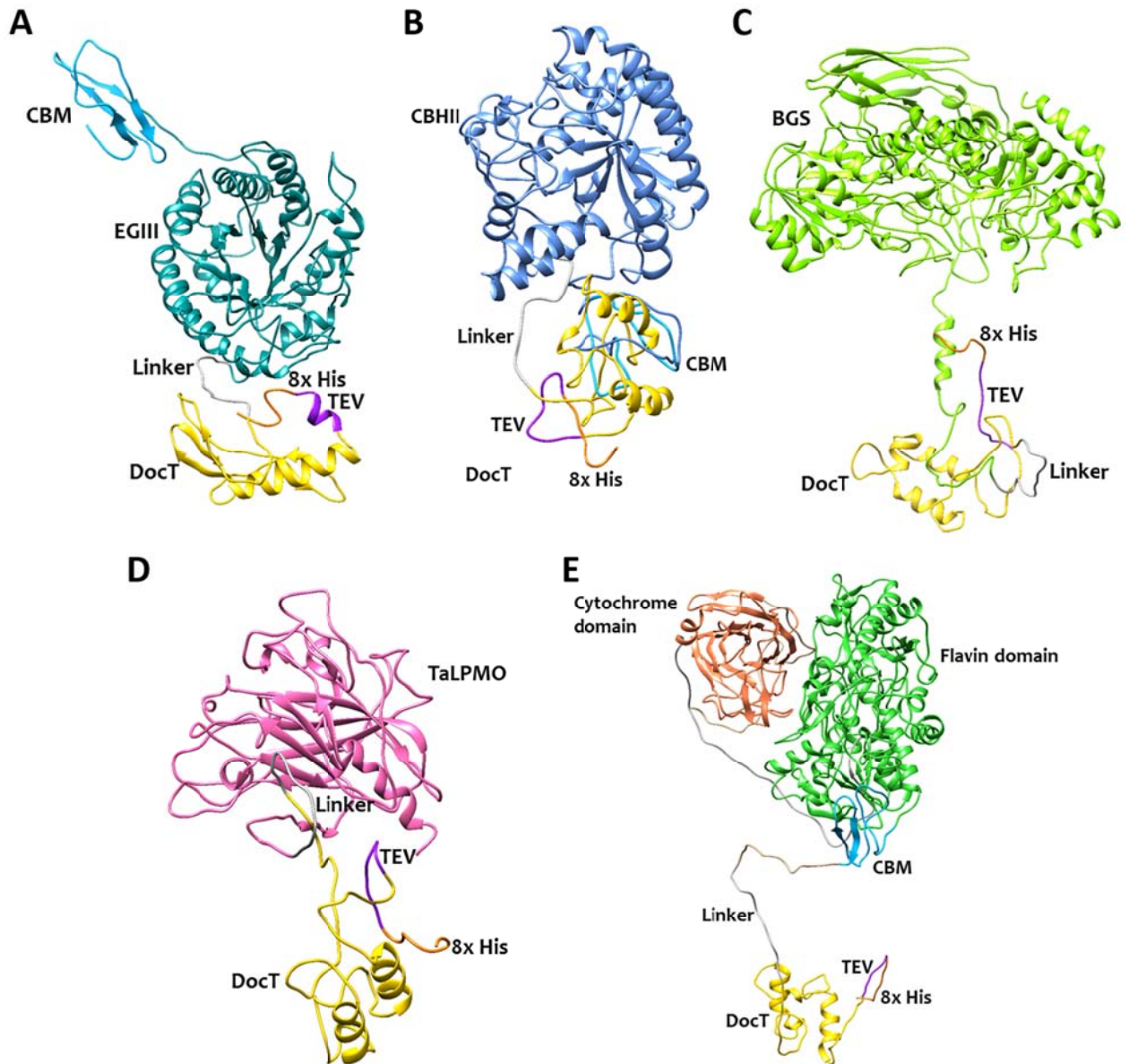


Fig. S4. Three-dimensional structures of dockerin fused enzymes predicted by homology modeling. The predicted structures of *TrEgIII-t* (a), *CBHII-t* (b), *NpaBGS-t* (c), *TaLPMO-t* (d) and *MtCDH-t* (e). The dockerin module is highlighted using golden color.

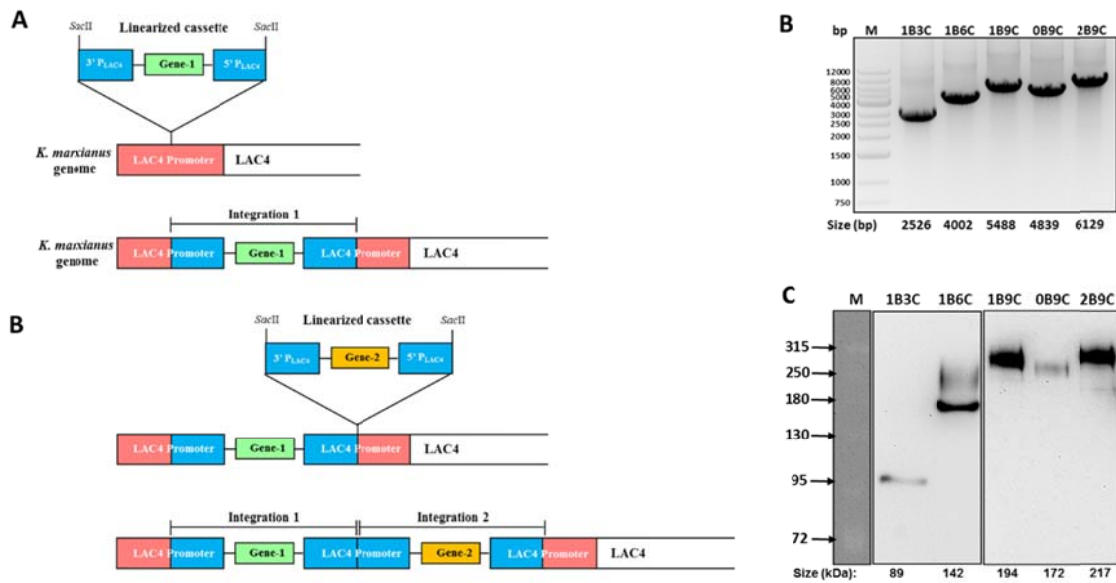


Fig. S5. Chromosomal integration of gene cassettes into LAC4 locus of the *Kluyveromyces marxianus* genome. **(A)** Single gene cassette integration. **(B)** Multiple gene cassette integration. Chromosomal integration and expression analysis of CipA variants. **(C)** Colony PCR analysis of CipA variants in *K. marxianus* chromosome. The genomic DNA was extracted using Quick Extract solution and used as a template for PCR verification. The size (bp) of each gene is given at the bottom of each lane. Each gene was amplified using the S1274-F and S1276-R primer pairs. **(D)** Western blot analysis of scaffoldin hosts and the anchoring host expressing CipA variants. The molecular weight of each protein is indicated at the bottom of each lane. The molecular weight of each CipA protein was slightly higher than the actual size, which might be due to the presence of multiple N-glycosylation sites in each CipA. For example, CipA1B3C and 1B6C each contain 4 potential N-glycosylation sites and CipA0B9C, 1B9C and 2B9C each contain 5 potential N-glycosylation sites. The N-glycosylation sites were predicted using NetNGlyc 1.0 Server (<http://www.cbs.dtu.dk/services/NetNGlyc/>)

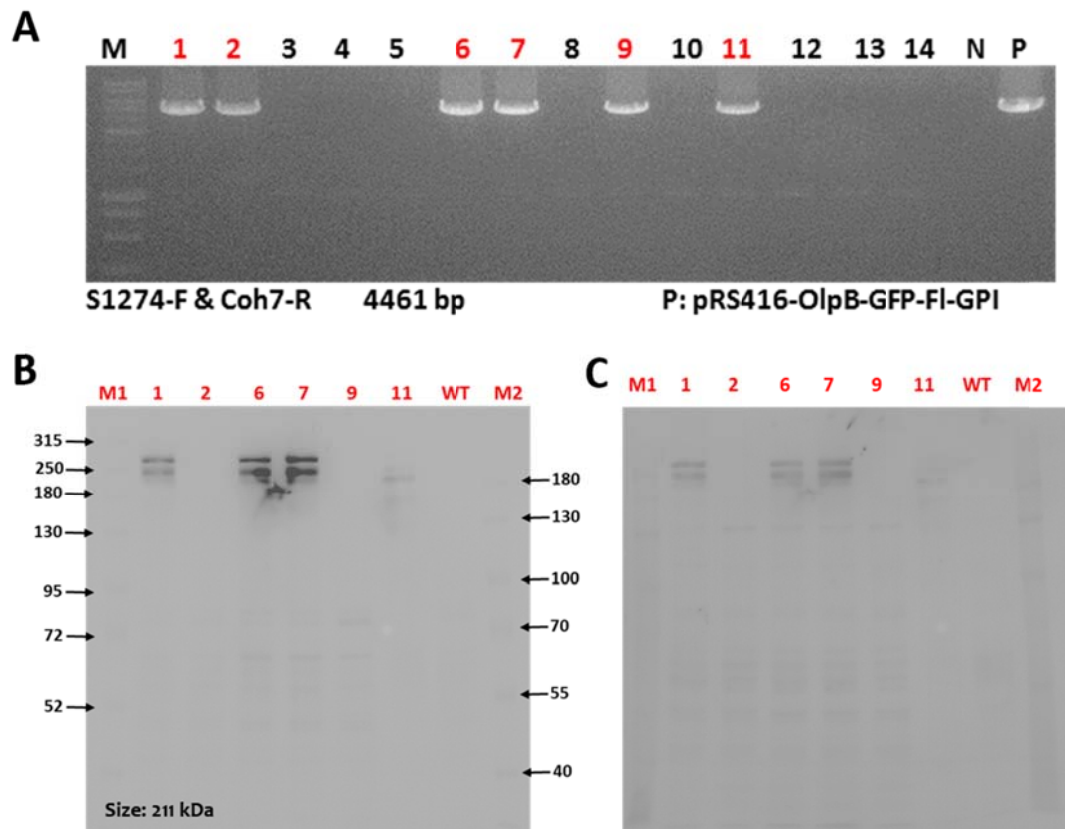


Fig. S6. Analysis of yeast cells expressing OlpB-ScGPI. (a) Colony PCR analysis using gene specific primers. (b) Western blot analysis of selected transformants using anti-His antibody. (c) Western blot analysis of selected transformants using anti-Flag antibody and the membrane after stripping.

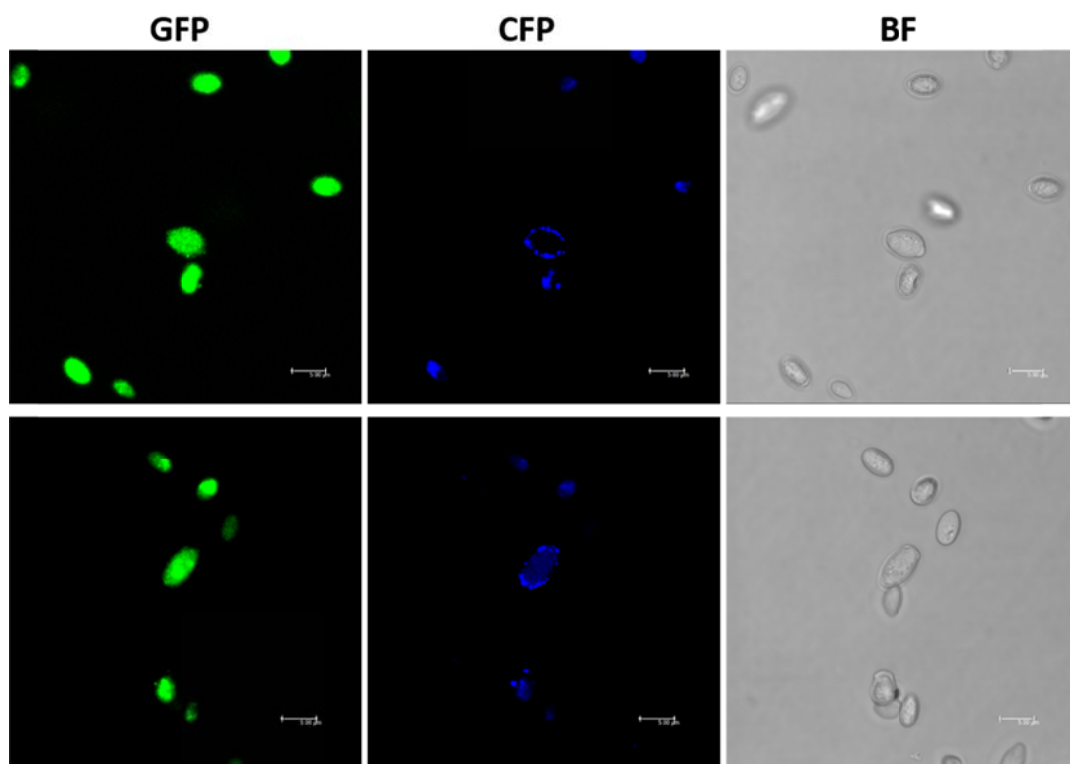


Fig. S7. Confirmation of OlpB-ScGPI anchoring on cell surface. Anchoring of OlpB-ScGPI on the *K. marxianus* cell surface confirmed by immunostaining and confocal microscopy using GFP or CFP.

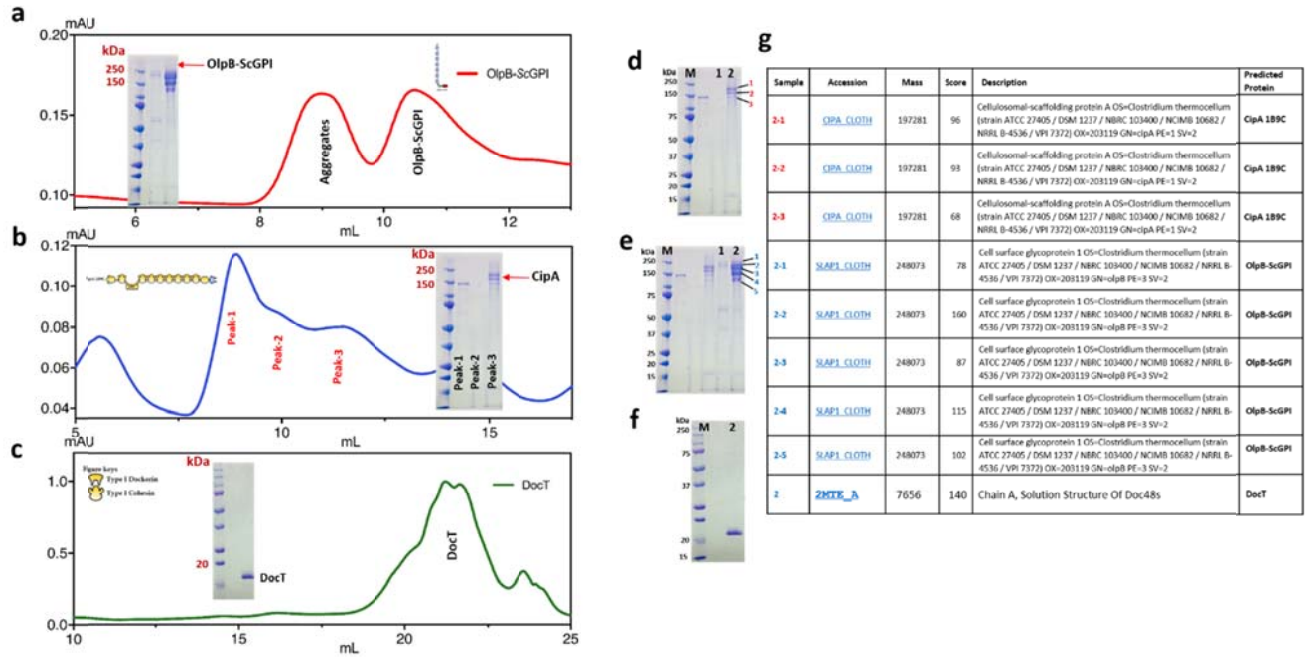
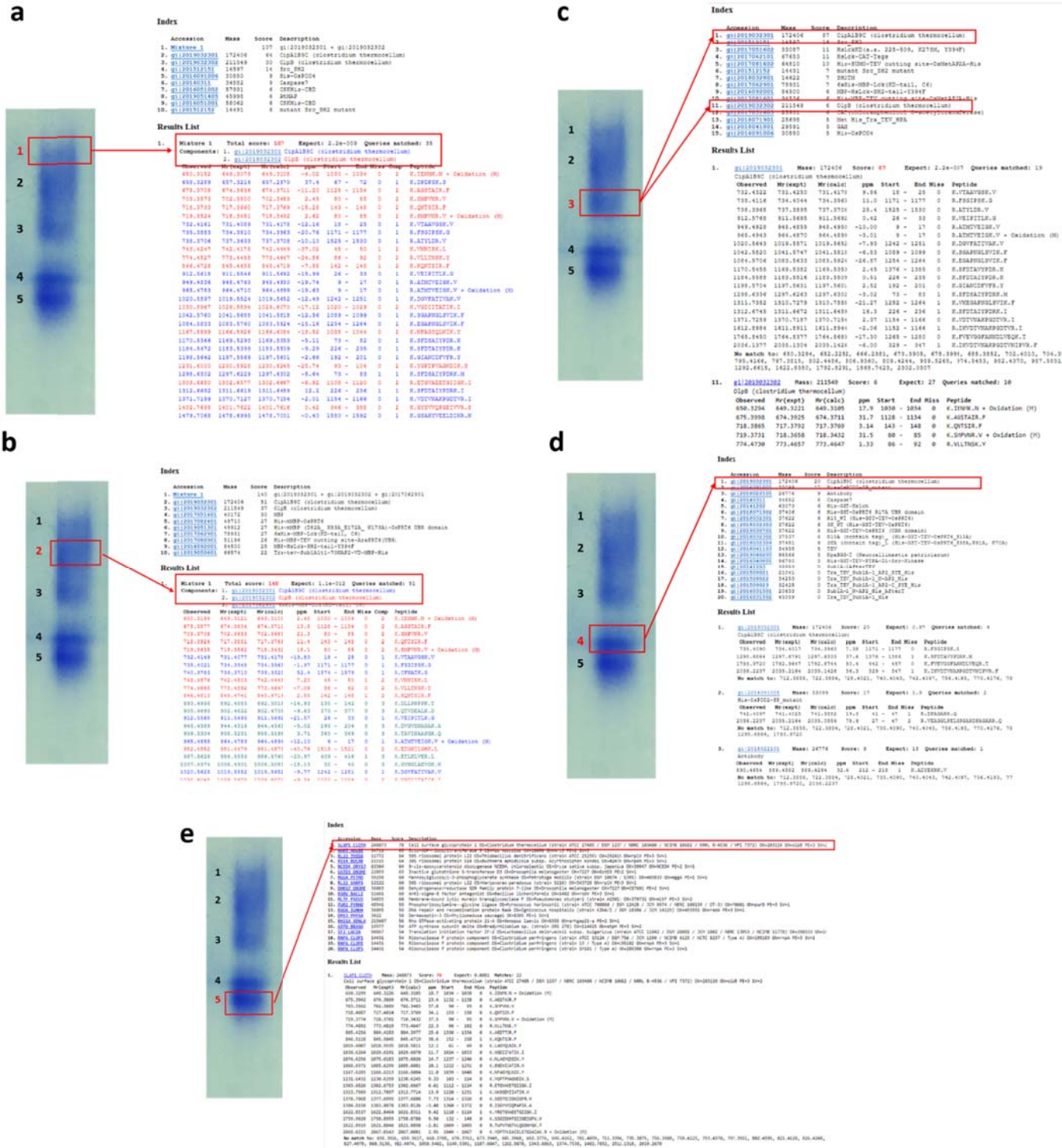


Fig. S8. Purification and confirmation of cellulosomal proteins. **a**, Schematic diagram of size-exclusion chromatography (SEC) of OlpB coloured in red. Chromatogram represents aggregates and monomeric soluble OlpB. Corresponding proteins were confirmed on 4-15% SDS-PAGE. **b**, The SEC purified CipA chromatogram illustrated in blue colour. The chromatogram possesses three peaks, among which peak 3 corresponds to the CipA molecular weight, and the resultants were confirmed by 4-15% SDS-PAGE. **c**, SEC chromatogram of DocT coloured in green and validation of the protein presence on 4-15% SDS-PAGE. **d-g**, Mass spectrometry identification of purified proteins. **d**, Three bands were observed at the CipA peak, all of which were identified as CipA. **e**, Five bands were observed at the OlpB peak, all of which were identified as OlpB. **g**, A single clear band was observed on DocT and was identified as DocT using mass spectrometry.



Fig. S9. Mass spectrometry identification of the CipA:DocT complex. The complex formation was conducted at 37°C for 3 hours with equimolar ratios of purified CipA and DocT. Then complex was further purified using S200 SEC column and fractions at peaks were analysed using 4-15 % SDS-PAGE. The observed two bands were analysed by mass spectrometry and identified as CipA and DocT.



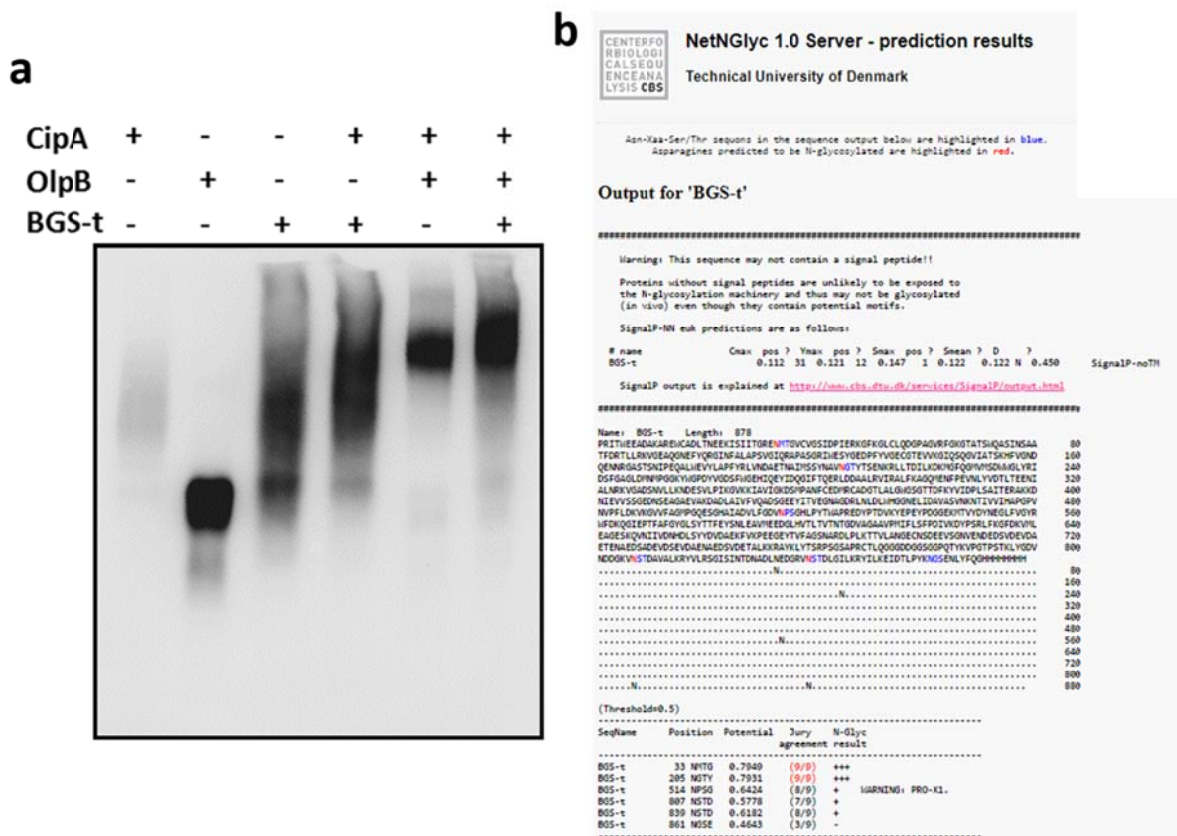


Fig. S11. Cellulosome complex formation analysis. **a**, Concentrated supernatants of CipA1B9C, *Npa*BGS-t, and cell lysate of OlpB-ScGPI were mixed together in different molar ratios and allowed to form complexes at 37°C with 10mM CaCl₂. The cellulosome complex was loaded into 4-15% native-PAGE and then western blot was performed using HRP-conjugated anti-His antibody. The migration of bands denotes the increased molecular weight, thus confirming the complex formation. **b**, N-Glycosylation prediction result of BGS-t using NetNGlyc 1.0 server (<http://www.cbs.dtu.dk/services/NetNGlyc/>)

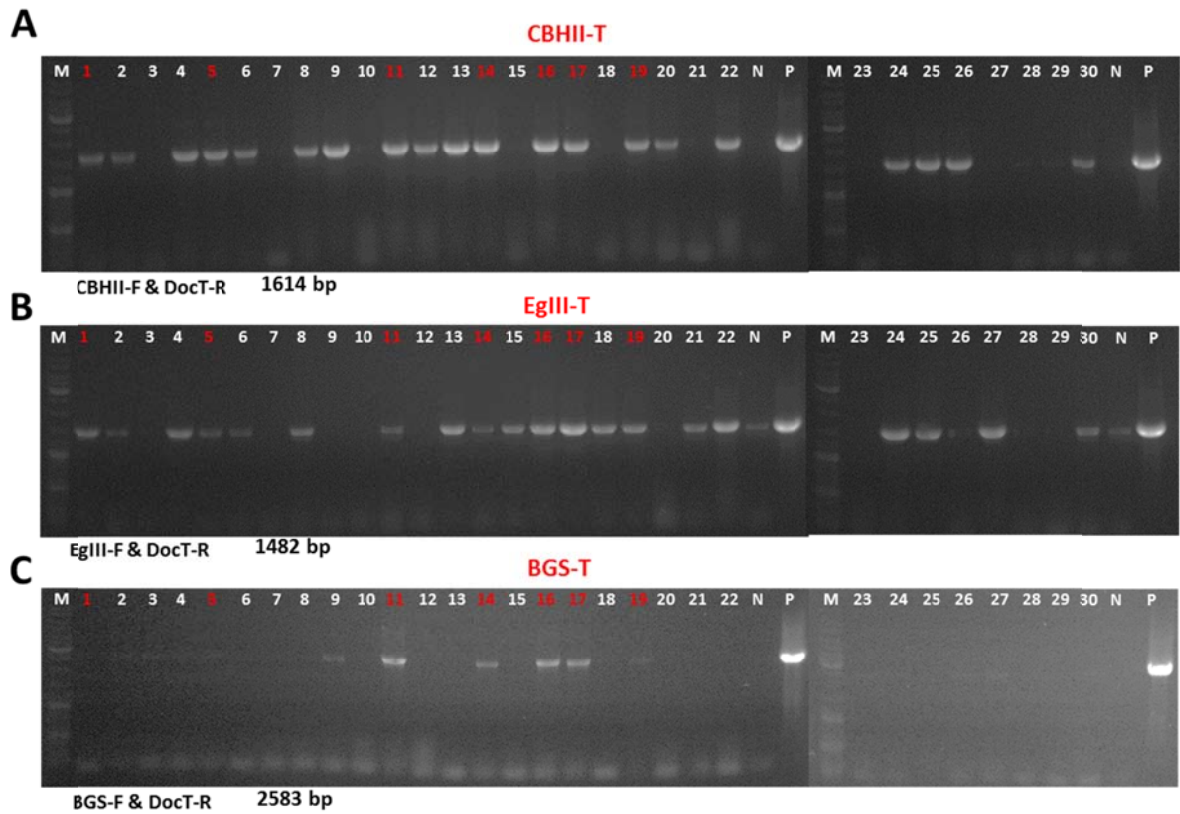


Fig. S12. PCR confirmation of the cellulase host (CH). Thirty colonies were randomly selected and sub-cultured for three generations to obtain stable transformants. The genomic DNA was extracted using Quick Extract solution and used as a template for PCR verification. The sizes (bp) of each gene and primer pairs are given at the bottom of each figure. Highlighted numbers are hosts with positive PCR confirmation of CBHII-t (a), EgIII-t (b) and BGS-t (c) and selected for activity assay. The primer sequences are listed in Supplementary Table 3.

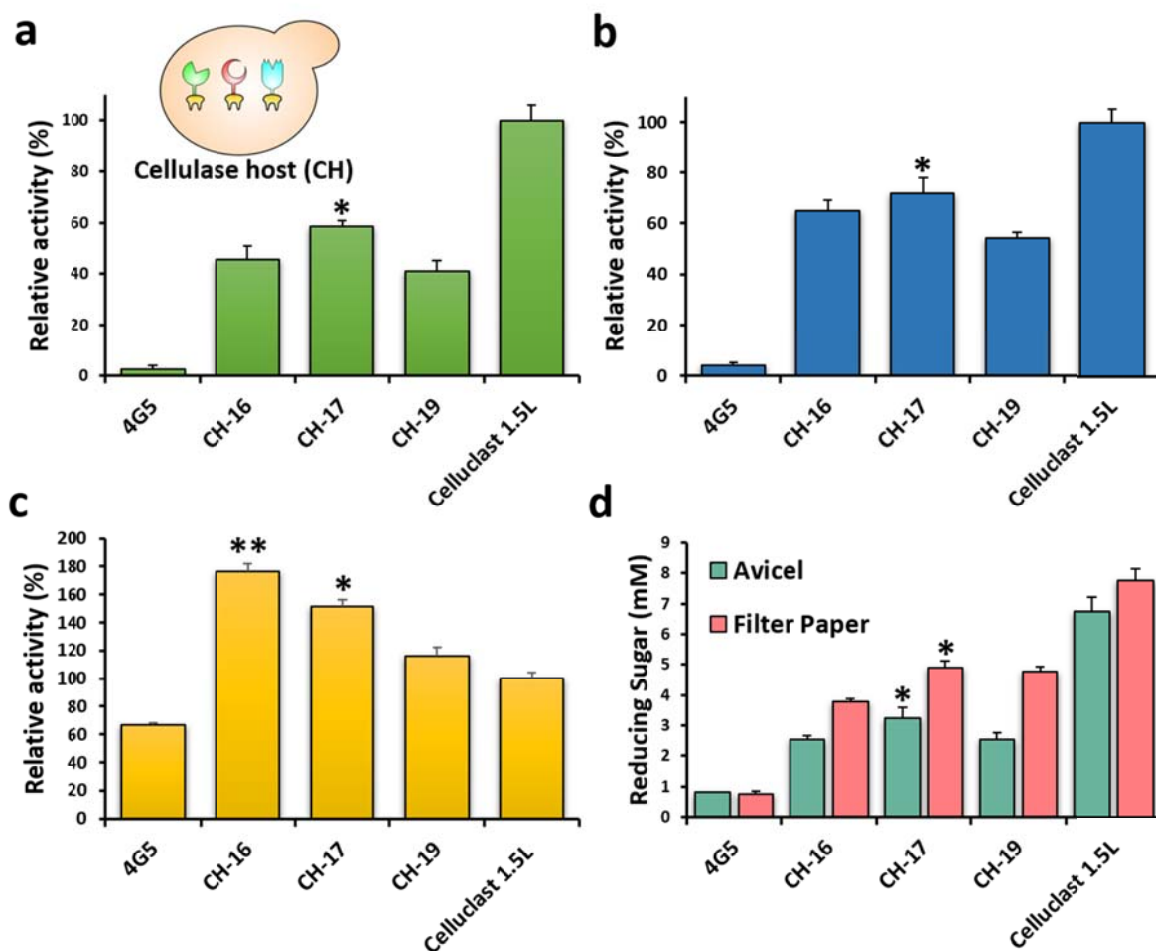


Fig. S13. Enzyme assay of the engineered cellulase host. Enzyme assays of cellulase hosts (CH) expressing *TrEGIII-t*, *CBHII-t* and *NpaBGS-t*. The numbers after CH represent the transformant numbers. **a**, CBH activity assay using PASC as the substrate. **b**, EG activity assay using CMC as the substrate. **c**, BGS activity assay using pNPG as the substrate. **d**, Soluble sugar release assay using avicel or filter paper as the substrate. For all enzyme assays, condensed culture supernatant of the CH host was used as an enzyme source and equal protein concentrations were calculated using BSA as a standard and used for enzyme assays. All enzyme assays were conducted at 40°C with 1200 rpm. The reducing sugar release was calculated using the DNS method. The results are expressed as mean (n=3) ± standard deviation (SD). * $P < 0.05$, ** $P < 0.01$; one-way ANOVA followed by Bonferroni post hoc test.

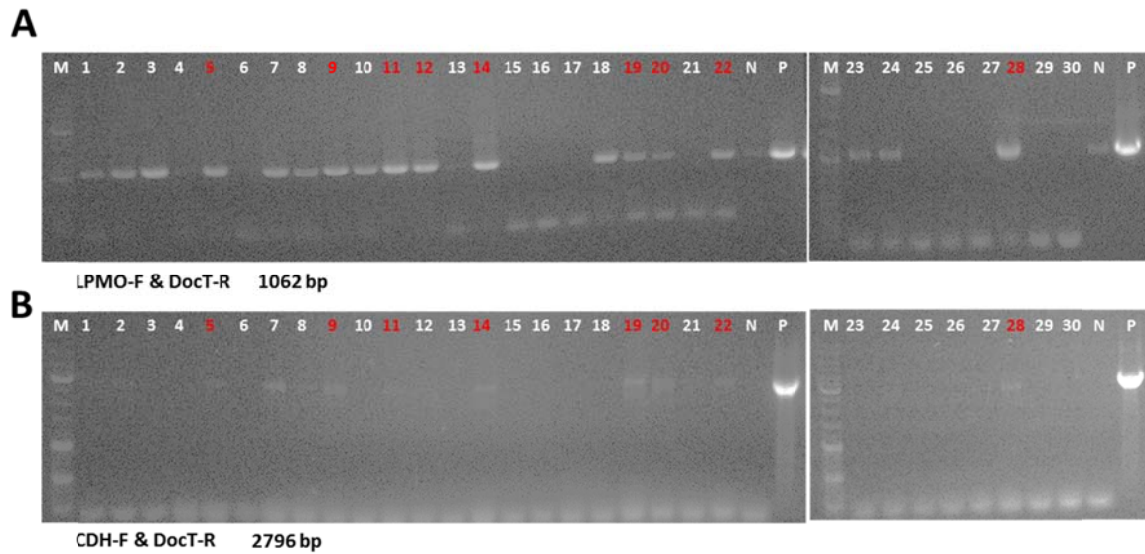


Fig. S14. PCR confirmation of the booster host (BH). Thirty colonies were randomly selected and sub-cultured for three generations to obtain stable transformants. The genomic DNA was extracted using Quick Extract solution and used as a template for PCR verification. The size (bp) of each gene and the primer pair are given at the bottom of each figure. Highlighted numbers are hosts with positive PCR confirmation of LPMO-t (**a**) and CDH-t (**b**) and selected for activity assays. The primer sequences are listed in Supplementary Table 3.

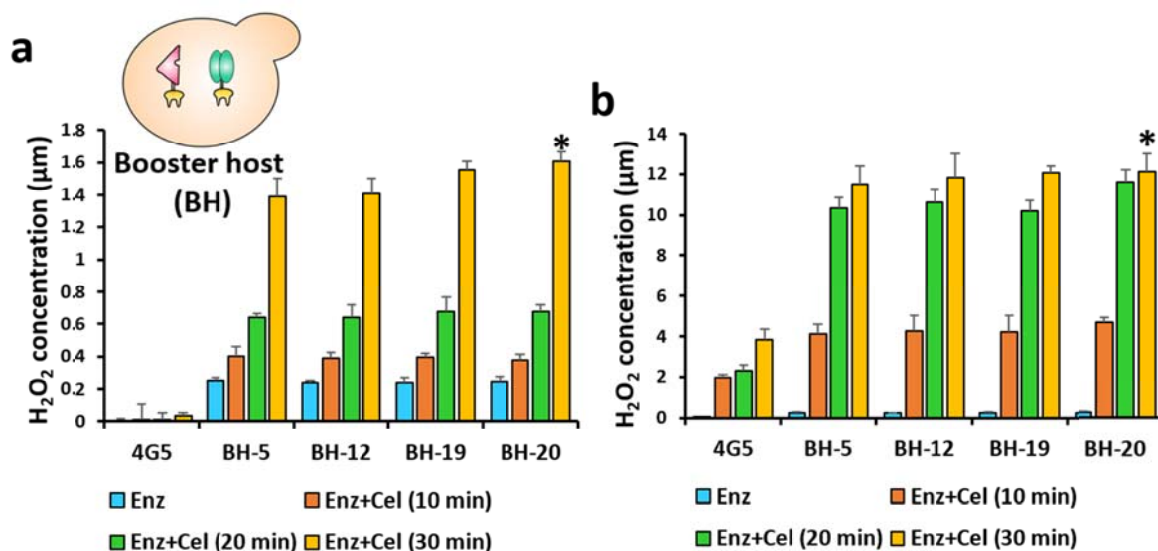


Fig. S15. Enzyme assays of the engineered booster host. Enzyme assays of booster hosts (BH) expressing *TaLPMO-t*, and *MtCDH-t*. The numbers after BH represent the transformant numbers. LPMO and CDH activity assays using cellobiose (**a**) or ascorbate (**b**) as the substrate for CDH, which donates electrons to LPMO. The H₂O₂ release was measured using the Amplex Red/HRP assay kit and Excitation/Emission was measured at 530/590 nm using fluorescence spectrophotometer. For all enzyme assays, condensed culture supernatant of the BH host was used as an enzyme source and equal protein concentrations were calculated using BSA as a standard and used for enzyme assays. All enzyme assays were conducted at 40°C with 1200 rpm. The results are expressed as mean (n=3) ± standard deviation (SD). **P*< 0.05, ***P*< 0.01; one-way ANOVA followed by Bonferroni post hoc test.

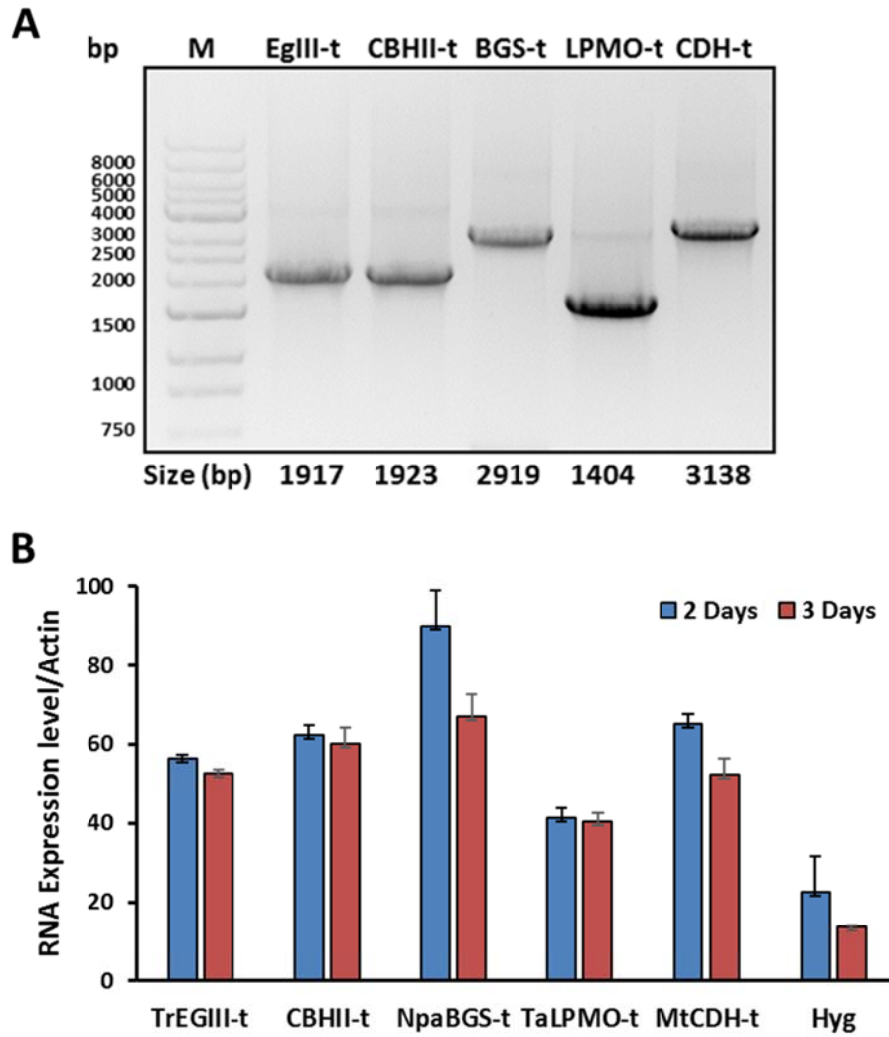


Fig. S16. Chromosomal integration and expression analysis of the enzyme host (EH). **a**, PCR confirmation of cellulosomal enzyme genes in the *K. marxianus* genome. The genomic DNA was extracted using Quick Extract solution and used as a template for PCR verification. The size (bp) of each gene is given at the bottom of each lane. Each gene was amplified using the Gene-F and Doct-R primer pairs. **b**, Quantitative PCR analysis of enzyme hosts. Relative mRNA expression levels of cellulase genes inserted into the EH host were calculated using Universal ProbeLibrary System (Roche). The relative expression ratio of each gene was calculated using the expression of the endogenous actin gene as the reference. The total RNA extraction was done using the yeast cells cultured for 3 days at 40°C.

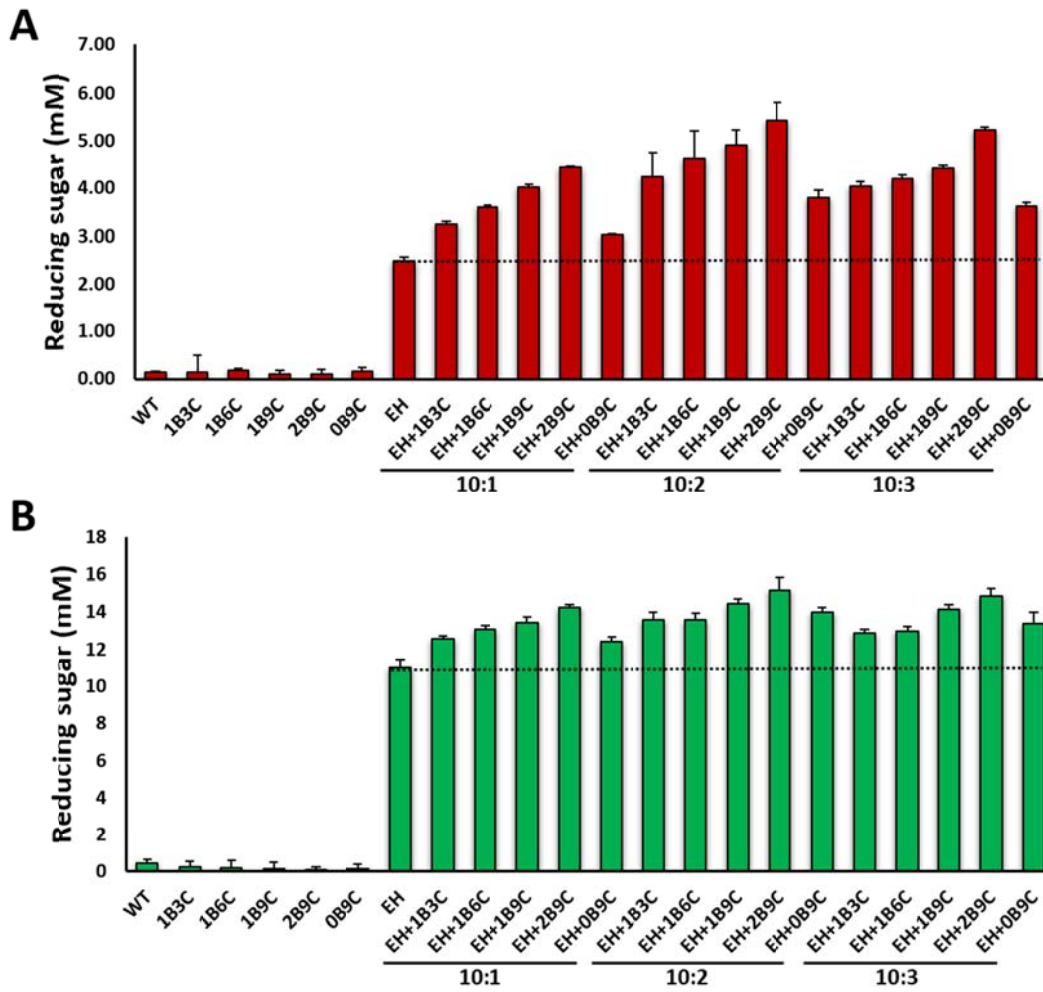


Fig. S17. Effects of CipA variants on avicel (a) or PASC (b) degradation. Enzyme host (EH) and CipA variants were cultured separately and condensed supernatants were used for the experiment. The enzyme variants and CipA were mixed in different ratios (10:1, 10:2 and 10:3) and allowed to form complexes, which were then mixed with avicel or PASC. The reducing sugar release was measured using the DNS method. The results are expressed as mean ($n=3$) \pm standard deviation (SD).

SI Appendix tables

Table S1: Bacterial and yeast strains used in this study

Strains	Genotype or relevant features	Reference/Source
Bacterial strains		
<i>Escherichia coli</i> DH5 α	F ⁻ Φ 80lacZ Δ M15 Δ (<i>lacZYA-argF</i>) U169 <i>recA1 endA1 hsdR17 (r_K, m⁺_K) phoA supE44 λ⁻</i> <i>thi-1 gyrA96 relA</i>	Real Biotech Corporation, Taiwan
<i>E. coli</i> JM110	<i>rpsL (Str^r) thr leu thi-1 lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) [F' traD36 proAB lacI^q ZΔM15]</i>	Stratagene, USA
<i>E. coli</i> BL21 His-MBP-DocT	<i>E. coli</i> BL21 carrying pET9a-His-MBP-DocT	This study
Yeast strains		
<i>Kluyveromyces marxianus</i> 4G5	Wild type strain isolate from kefir grains	Lee et al., 2018(1)
<i>K. marxianus</i> CH-17	Chromosomally integrated with three cellulases (CBHII-t, TrEgIII-t, and NpaBGS-t). *SM: Kanamycin	This study
<i>K. marxianus</i> BH-20	Chromosomally integrated with cellulase booster and its enzyme partner (<i>Ta</i> LPMO-t and <i>Mt</i> CDH-t). SM: Hygromycin	This study
<i>K. marxianus</i> EH-P1-44	Chromosomally integrated with three cellulases, cellulase booster and its enzyme partner (CBHII-t, TrEgIII-t, NpaBGS-t, <i>Ta</i> LPMO-t and <i>Mt</i> CDH-t). SM: Kanamycin and Hygromycin	This study
<i>K. marxianus</i> CipA1B3C	Chromosomally integrated with CipA1B3C. SM: Hygromycin	This study
<i>K. marxianus</i> CipA1B6C	Chromosomally integrated with CipA1B6C. SM: Hygromycin	This study
<i>K. marxianus</i> CipA1B9C	Chromosomally integrated with CipA1B9C. SM: Hygromycin	This study
<i>K. marxianus</i> CipA0B9C	Chromosomally integrated with CipA0B9C. SM: Hygromycin	This study
<i>K. marxianus</i> CipA2B9C	Chromosomally integrated with CipA2B9C. SM: Hygromycin	This study
<i>K. marxianus</i> OlpB-ScGPI	Chromosomally integrated with OlpB-ScGPI. SM: Kanamycin and Hygromycin	This study
<i>K. marxianus</i> SH-0B9C	Chromosomally integrated with OlpB-ScGPI and CipA0B9C. SM: Kanamycin and Hygromycin	This study
<i>K. marxianus</i> SH-1B9C	Chromosomally integrated with OlpB-ScGPI and CipA1B9C. SM: Kanamycin and Hygromycin	This study
<i>K. marxianus</i> SH-2B9C	Chromosomally integrated with OlpB-ScGPI and CipA2B9C. SM: Kanamycin and Hygromycin	This study
<i>K. marxianus</i> RFP-DocT	Chromosomally integrated with RFP-DocT. SM: Hygromycin	This study

*SM: Selection marker

Table S2: Fungal cellulases used in this study.

Gene	Enzyme family	Source	Host species	Reference
<i>TrEgIII</i>	GH5 Endoglucanase	<i>Trichoderma reesei</i> ATCC 13631	fungus	Chang et al, 2012(2)
CBHII	GH6 Cellobiohydrolase	Synthetic gene	fungus	Chang et al, 2013(3)
<i>NpaBgs</i>	GH3 Beta-glucosidase	<i>Neocallimastix patriciarum</i> W5	fungus	Chen et al, 2012(4)
<i>TaLMPO</i>	AA9 Lytic polysaccharide monooxygenase	<i>Thermoascus aurantiacus</i>	fungus	Harris et al., 2010(5)
<i>MtCDH</i>	CBM1 Cellobiose dehydrogenase	<i>Myceliophthora thermophila</i> ATCC 42464	fungus	Phillips et al., 2011(6)

Table S3: Primer pairs used in this study for cloning and PCR verification

Name	Sequence (5' to 3')	Purpose
<i>TrEgIII-AvrII-F</i>	ATTCTAGGCAGCAGACTGTCTGG	Dockerin fusion
<i>TrEgIII-SpeI-R</i>	ATGACTAGTCTTTCTTGCAGACACG	Dockerin fusion
<i>CBHII-AvrII-F</i>	ATGCCTAGGCAACAACTTTGTGGGGT	Dockerin fusion
<i>CBHII-SpeI-R</i>	ATTACTAGTGAAGGCTGGGTAGCGTTA	Dockerin fusion
<i>NpaBGS-AvrII-F</i>	ATACCTAGGATTACTTGGGAAGAAG	Dockerin fusion
<i>NpaBGS-SpeI-R</i>	GAGACTAGTGTAAGTTTGTAAGCT	Dockerin fusion
<i>TaLPMO-AvrII-F</i>	GGTGGCCTAGGTCCTTCTCTAAGATTATT	Dockerin fusion
<i>TaLPMO-SpeI-R</i>	GATCACTAGTACCGGTGTACAATGGTGGA	Dockerin fusion
<i>MtCDH-AvrII-F</i>	ATTGCCTAGGAGAACCTCCTCCAGAT	Dockerin fusion
<i>MtCDH-SpeI-R</i>	GCAGACTAGTCAAACATTGGGAGTACC	Dockerin fusion
S1274-F	GCGGATAACAAGCTCAAC	PCR Verification
S1276-R	TCGGCACTAATAACCGTT	PCR Verification
DocT-R	GTTCTTGTACGGCAATGTATCTATTTC	PCR Verification
pK-CipA1B9C-F	CCTAGGGCCACCATGACTGT	PCR Verification
pK-CipA1B9C-R	CTGGGCGTCGTAATCACTGCT	PCR Verification
pK-CipA1B6C-F	GCAACAATGACCGTCGAAATCG	PCR Verification
pK-CipA1B6C-R	CTGGGCGTCGTAGTCACTG	PCR Verification
pK-CipA1B3C-F	GCAACCATGACAGTGGAATA	PCR Verification
pK-CipA1B3C-R	TTATTGTGCATCATAATCAGA	PCR Verification
OlpB-Coh1-F	ATTGAAATGGTCCTGGATAA	PCR Verification
OlpB-Coh7-R	ACTGGCAGCTTTGATAGGTGCT	PCR Verification
GFP-F	AACCTAGGACGCGTGTGAGCAAGGGC	PCR Verification
<i>ScGPI-R</i>	GGCGCGGCCGCTTAATTAATTAGAATAGC	PCR Verification

F: Forward primers R: Reverse primers

Table S4: Primer pairs used for the qPCR analysis

Name*	Sequence (5' to 3')
EgIII-T#25-F	TCGTGTCTCGCAAGAAAGACT
EgIII-T#25-R	CTTGAGGACCACCGCTTC
CBHII-T#25-F	ACGCTAACCCAGCCTTCAC
CBHII-T#25-R	CTTGAGGACCACCGCTTC
BGS-T#150F	GAAGCTGTAATGGAAGAAGATGG
BGS-T#150R	CTGGGAATGAAAGGAAAATCAT
<i>Ta</i> LPMO-T#113-F	TGGTAACTACGTCTTGAGACACG
<i>Ta</i> LPMO-T#113-R	GTTTTGAGCACCGTCTTGGT
<i>Mt</i> CDHIIA-T#109.154-F	GAATTTTGGCTTTGCCAGAC
<i>Mt</i> CDHIIA-T#109.154-R	GGACCATTCTCTACCACCACA
CipA1B3C#128-F	GAGATCTCCTTTACCGGTGGT
CipA1B3C#128-R	AACCTGCCTTGAATCTGCAC
CipA1B6C#80.116-F	TGACCCGAACGTGTTAGAGA
CipA1B6C#80.116-R	TGTCCGGATTAGGATCGACT
CipA1B9C#116-F	CCCCAATGTGCTCGAGATAA
CipA1B9C#116-R	GTGGGGTTTGGATCAACAAT
CipA2B9C#77.87-F	CACCCGCTACGACAAAGC
CipA2B9C#77.87-R	ACGGCGGGATAGTTGTTG
CipA0B9C#57-F	TCTGTTTGCAGAGGACTCAGG
CipA0B9C#57-R	GCGAACACACCGTCTTCTG
OlpB- <i>Sc</i> GPI#15.32-F	AGACAACGGCCGTAGCAA
OlpB- <i>Sc</i> GPI#15.32-R	CTGTAGGCTTCAGCAAATTGA
KanMX#144-F	AGACTAAACTGGCTGACGGAAT
KanMX#144-R	CATCAGGAGTACGGATAAAATGC
HygB#143-F	GGGATTCCCAATACGAGGTC
HygB#143-R	GCTCCATACAAGCCAACCAC
ACTIN#9-F	GCGTAGATTGGAACAACGTG
ACTIN#9-R	AGAACTACCGGTATTGTGTTGGA

*Numbers after # denotes the UPL probes used for the analysis.

Table S5: Amino acid sequences of cellulosomal genes used in this study

Gene name	Amino acid sequence
Type I dockerin (DocT) of celS from <i>C. thermocellum</i>	TYKVPSTKLYGDVNDGKVNSTDAVALKRYVLRSGISINTDNADLNEDGRVNSTDLGILKRYILKEIDTLPLYKN
CipA1B3C (89 kDa)	HHHHHHPRATMTVEIGKVTAAVGSKVEIPITLKGVPKGMANCDVFLGYDPNVLEVTEVKPGSIKDPDPSKSFDSAIPDRKMIVFLFAEDSGRGTAYITQDGVFATIVATVKSAAAAPITLLEVGAFADNDLVEI STTFVAGGVNLGSSVPTTQPNVPSDGVVVEIGKVTGSGVTTVEIPVYFRGVPSKGIANCDVFRYDPN VLEIIGIDPGDIIVDPNPTKSFDTAIYDPRKIIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITFD EVGGFADNDLVEQKVSFIDGGVNVGNATPTKCATPTNTATPTKSATATPTRPSVPTNTPTNTPTANTPV SGNLKVEFYNSNPSTTNSINPQFKVTNTGSSAIDLKSLTLRYYYTVDGQKQDQTFWCDHAAIIGSNGS YNGITSNVKGTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSNDSYFKSAS QFVEWDQVTA YLNGVLVWGKEPGGSVVPSTQPVTPPATTKPPATTKPPATTIPPSDDPNAIKIKVDT VNAKPGDVTNIPVRFSGIPSKGIANCDVFSYDNPVLEIIEIKPGEIVDPNPKSFDTA VYPDRKIIIVFL FAEDSGTGAYAITKDGVFATIVAKVKS GAPNGLSVIKFVEVGGFANNDLVEQRTQFFDGGVNVGDN KPVIIEGYKVS GYILPDFSFDATVAPLVKAGFKVEIVGTELYAVTDANGYFEITGVPANASGYTLKISRA TYLDRVIANVVVTGDTSVSTSQAPIMMWVGDIVKDNSINLLDVAEIVRCFNATKGSANYVEELDINR NGAINMQDIMIVHKHFGATSSDYDAQ
CipA1B6C (142 kDa)	HHHHHHPRATMTVEIGKVTAAVGSKVEIPITLKGVPKGMANCDVFLGYDPNVLEVTEVKPGSIKDPDPSKSFDSAIPDRKMIVFLFAEDSGRGTAYITQDGVFATIVATVKSAAAAPITLLEVGAFADNDLVEI STTFVAGGVNLGSSVPTTQPNVPSDGVVVEIGKVTGSGVTTVEIPVYFRGVPSKGIANCDVFRYDPN VLEIIGIDPGDIIVDPNPTKSFDTAIYDPRKIIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITFD EVGGFADNDLVEQKVSFIDGGVNVGNATPTKCATPTNTATPTKSATATPTRPSVPTNTPTNTPTANTPV SGNLKVEFYNSNPSTTNSINPQFKVTNTGSSAIDLKSLTLRYYYTVDGQKQDQTFWCDHAAIIGSNGS YNGITSNVKGTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSNDSYFKSAS QFVEWDQVTA YLNGVLVWGKEPGGSVVPSTQPVTPPATTKPPATTKPPATTIPPSDDPNAIKIKVDT VNAKPGDVTNIPVRFSGIPSKGIANCDVFSYDNPVLEIIEIKPGEIVDPNPKSFDTA VYPDRKIIIVFL FAEDSGTGAYAITKDGVFATIVAKVKS GAPNGLSVIKFVEVGGFANNDLVEQRTQFFDGGVNVGDTT VPTTPTPVTTPTDSSNAVRIKVDVTNAPKPGDTRIPVRFSGIPSKGIANCDVFSYDNPVLEIIEIEPGD IIVDPNPKSFDTA VYPDRKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKS GAPNGLSVIKFVEVGGF ANNDLVEQKTQFFDGGVNVGDTTEPATPTTPVTTPTTTDDLDVAVRIKVDVTNAPKPGDTRIPVRFSGI PSKGIANCDVFSYDNPVLEIIEIEPGDIIVDPNPKSFDTA VYPDRKIIIVFLFAEDSGTGAYAITKDGVF ATIVAKVKS GAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTTPVTTPTTTDDLD DAVRIKVDVTNAPKPGDTRIPVRFSGIPSKGIANCDVFSYDNPVLEIIEIEPGDIIVDPNPKSFDTA V YPDRKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKS GAPNGLSVIKFVEVGGFANNDLVEQKTQFFD GGVNVGNKPVIEGYKVS GYILPDFSFDATVAPLVKAGFKVEIVGTELYAVTDANGYFEITGVPANAS GYTLKISRATY LDRVIANVVVTGDTSVSTSQAPIMMWVGDIVKDNSINLLDVAEIVRCFNATKGSAN YVEELDINRNGAINMQDIMIVHKHFGATSSDYDAQ
CipA1B9C (194 kDa)	HHHHHHPRATMTVEIGKVTAAVGSKVEIPITLKGVPKGMANCDVFLGYDPNVLEVTEVKPGSIKDPDPSKSFDSAIPDRKMIVFLFAEDSGRGTAYITQDGVFATIVATVKSAAAAPITLLEVGAFADNDLVEI STTFVAGGVNLGSSVPTTQPNVPSDGVVVEIGKVTGSGVTTVEIPVYFRGVPSKGIANCDVFRYDPN VLEIIGIDPGDIIVDPNPTKSFDTAIYDPRKIIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITFD EVGGFADNDLVEQKVSFIDGGVNVGNATPTKCATPTNTATPTKSATATPTRPSVPTNTPTNTPTANTPV SGNLKVEFYNSNPSTTNSINPQFKVTNTGSSAIDLKSLTLRYYYTVDGQKQDQTFWCDHAAIIGSNGS YNGITSNVKGTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSNDSYFKSAS QFVEWDQVTA YLNGVLVWGKEPGGSVVPSTQPVTPPATTKPPATTKPPATTIPPSDDPNAIKIKVDT VNAKPGDVTNIPVRFSGIPSKGIANCDVFSYDNPVLEIIEIKPGEIVDPNPKSFDTA VYPDRKIIIVFL FAEDSGTGAYAITKDGVFATIVAKVKS GAPNGLSVIKFVEVGGFANNDLVEQRTQFFDGGVNVGDTT VPTTPTPVTTPTDSSNAVRIKVDVTNAPKPGDTRIPVRFSGIPSKGIANCDVFSYDNPVLEIIEIEPGD IIVDPNPKSFDTA VYPDRKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKS GAPNGLSVIKFVEVGGF ANNDLVEQKTQFFDGGVNVGDTTEPATPTTPVTTPTTTDDLDVAVRIKVDVTNAPKPGDTRIPVRFSGI PSKGIANCDVFSYDNPVLEIIEIEPGDIIVDPNPKSFDTA VYPDRKIIIVFLFAEDSGTGAYAITKDGVF ATIVAKVKS GAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTTPVTTPTTTDDLD DAVRIKVDVTNAPKPGDTRIPVRFSGIPSKGIANCDVFSYDNPVLEIIEIEPGDIIVDPNPKSFDTA V YPDRKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKS GAPNGLSVIKFVEVGGFANNDLVEQKTQFFD GGVNVGDTTEPATPTTPVTTPTTTDDLDVAVRIKVDVTNAPKPGDTRIPVRFSGIPSKGIANCDVFSY DNPVLEIIEIEPGELIVDPNPTKSFDTA VYPDRKMIVFLFAEDSGTGAYAITEDGVFATIVAKVKS GAPN GLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTTPVTTPTTTDDLDVAVRIKVDVTNAPK PGDTRIPVRFSGIPSKGIANCDVFSYDNPVLEIIEIEPGDIIVDPNPKSFDTA VYPDRKIIIVFLFAEDS GTGAYAITKDGVFATIVAKVKS GAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTVPPTS PTTTPEPTITPNKLT LKIGRAEGRPGDVEIPVNLVGVVQKGIASGDFVVSYDNPVLEIIEIEPGELIVDP NPTKSFDTA VYPDRKMIVFLFAEDSGTGAYAITEDGVFATIVAKVKS GAPNGLSVIKFVEVGGFANNDL VEVETDLINGGVLVTNKPVIIEGYKVS GYILPDFSFDATVAPLVKAGFKVEIVGTELYAVTDANGYFEI TGVPANASGYTLKISRATY LDRVIANVVVTGDTSVSTSQAPIMMWVGDIVKDNSINLLDVAEIVRCFN ATKGSANYVEELDINRNGAINMQDIMIVHKHFGATSSDYDAQ
CipA0B9C (172 kDa)	HHHHHHPRATMTVEIGKVTAAVGSKVEIPITLKGVPKGMANCDVFLGYDPNVLEVTEVKPGSIKDPDPSKSFDSAIPDRKMIVFLFAEDSGRGTAYITQDGVFATIVATVKSAAAAPITLLEVGAFADNDLVEI STTFVAGGVNLGSSVPTTQPNVPSDGVVVEIGKVTGSGVTTVEIPVYFRGVPSKGIANCDVFRYDPN VLEIIGIDPGDIIVDPNPTKSFDTAIYDPRKIIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITFD EVGGFADNDLVEQKVSFIDGGVNVGDTTEPATPTTPVTTPTTTDDLDNAIKIKVDVTNAPKPGDVTNIP VRFSGIPSKGIANCDVFSYDNPVLEIIEIKPGEIVDPNPKSFDTA VYPDRKIIIVFLFAEDSGTGAYAI

	<p>TKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQRTQFFDGGVNVGDTTPTPTPTPTPT TDDSNVRIKVDVTNNAKPGDTRIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIEPGDIIVDPNPDKSFD TAVYPDRKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTQ FFDGGVNVGDTTEPATPTPTPTPTTTDDLDVARIKVDVTNNAKPGDTRIPVRFSGIPSKGIANCDFVY SYDPNVLEIIEIEPGDIIVDPNPDKSFD TAVYPDRKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKSGAP NGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTPTPTPTTTDDLDVARIKVDVTNNA KPGDTRIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIEPGDIIVDPNPDKSFD TAVYPDRKIIIVFLFAED SGTGAYAITKDGVFATIVAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPAT PTPTPTPTTTDDLDVARIKVDVTNNAKPGDTRIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIEPGELI VDPNPDKSFD TAVYPDRKMIVFLFAEDSGTGAYAITEDGVFATIVAKVKSGAPNGLSVIKFVEVGGFA NNDLVEQKTQFFDGGVNVGDTTEPATPTPTPTPTTTDDLDVARIKVDVTNNAKPGDTRIPVRFSGIP SKGIANCDFVYSYDPNVLEIIEIEPGDIIVDPNPDKSFD TAVYPDRKIIIVFLFAEDSGTGAYAITKDGVFA TIVAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTVPPTSPTTTTPEPTITPNKLT LKIGRAEGRPGDVEIPVNLVYGVVQKGIASGDFVYSYDPNVLEIIEIEPGELIVDPNPDKSFD TAVYPDR KMIVFLFAEDSGTGAYAITEDGVFATIVAKVKEGAPEGFSAIESEFGAFADNDLVEVETDLINGGVLV TNKPVIEGYKVSGLPDFSFDATVAPLVKAGFKVEIVGTELYAVTDANGYFEITGVPANASGYTLKIS RATYLDRIANVVVVGDTSVSTSQAPIMMWVGDIVKDNSINLLDVAEIVRCFNATKGSANYVEELDI NRNGAINMQDIMIVHKHFGATSSDYDAQ</p>
<p>CipA2B9C (217 kDa)</p>	<p>HHHHHHHPRATMTVEIGKVTAAVGSKEIPIITLKGVPKGMANCDFVLGYDPNVLEVTEVKPGSIKDP DPSKSFDSAIPDRKMIVFLFAEDSGRGTAYAITQDGVFATIVATVKSAAAAPITLLEVGAFADNDLVEI YTFVAGVNLGSSVPTTQPNVPSDGVVVEIGKVTGVSQTTVEIPVYFRGVPKGMANCDFVYSYDPNVLEI VLEIIGIDPGDIIVDPNPDKSFD TAVYPDRKIIIVFLFAEDSGTGAYAITKDGVFAKIRATVKSAPGYITFD EVGGFADNDLVEQKVSFIDGGVNVGNATPTKATPTNTATPTKSATATPTRPSVPTNTPTNTANTPTV SGNLKVEFYNSNPSTTNSINPQFKVTNTGSSAIDLKSLTLRYYYTVDGGKQDQTFWCDHAAIIGSNGS YNGITSNVKGTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSDNYFVDPN QFVEWDQVTA YLNGVLVWGKEPGGSVVSTQPVTPPATTKPPATTKPPATTPPSDDPNAIKIKVD VNAKPGDTRIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIEPGELIVDPNPDKSFD TAVYPDRKIIIVFL FAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQRTQFFDGGVNVGDTT VPTPTPTPTTDDSNVRIKVDVTNNAKPGDTRIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIEPGD IIVDPNPDKSFD TAVYPDRKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGF ANNDLVEQKTQFFDGGVNVGDTTEPATPTPTPTPTTTDDLDVARIKVDVTNNAKPGDTRIPVRFSGI PSKIANCDFVYSYDPNVLEIIEIEPGDIIVDPNPDKSFD TAVYPDRKIIIVFLFAEDSGTGAYAITKDGVF ATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTEPATPTPTPTPTTTDDLD DAVRIKVDVTNNAKPGDTRIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIEPGDIIVDPNPDKSFD TAV YPDRKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFD GGVNVGDTTEPATPTPTPTPTTTDDLDVARIKVDVTNNAKPGDTRIPVRFSGIPSKGIANCDFVYSY DPNVLEIIEIEPGELIVDPNPDKSFD TAVYPDRKMIVFLFAEDSGTGAYAITEDGVFATIVAKVKSGAPN GLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGNATPTKATPTNTATPTKSATATPTRPSVPTNTPT NTPANTPVSGNLKVEFYNSNPSTTNSINPQFKVTNTGSSAIDLKSLTLRYYYTVDGGKQDQTFWCDH AAIIGSNGSYNGITSNVKGTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSN DYSFKSASQFVEWDQVTA YLNGVLVWGKEPGGSVVSTQPVTPPATTKPPATTKPPATTPPSDDPA VRIKVDVTNNAKPGDTRIPVRFSGIPSKGIANCDFVYSYDPNVLEIIEIEPGDIIVDPNPDKSFD TAVYP RKIIIVFLFAEDSGTGAYAITKDGVFATIVAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGV NVGDTTVPPTSPTTTPEPTITPNKLT LKIGRAEGRPGDVEIPVNLVYGVVQKGIASGDFVYSYDPNV LEIIEIEPGELIVDPNPDKSFD TAVYPDRKMIVFLFAEDSGTGAYAITEDGVFATIVAKVKEGAPEGFSAIE SEFGAFADNDLVEVETDLINGGVLV TNKPVIEGYKVSGLPDFSFDATVAPLVKAGFKVEIVGTELY AVTDANGYFEITGVPANASGYTLKISRATYLDRIANVVVVGDTSVSTSQAPIMMWVGDIVKDNSIN LLDVAEIVRCFNATKGSANYVEELDIRNGAINMQDIMIVHKHFGATSSDYDAQ</p>
<p>OlpB-ScGPI (211 kDa)</p>	<p>HHHHHHHHHHHHSAGTTREATPSIEMVLDKTEVHVGDVITATIKVNNIRKLAGYQLNIKFDPEVLQP VDPATGEEFTDKSMPVNRVLLTNSKYGPTPVAGNDIKSGIINFATGYNNLTA YKSSGAIDEHTGIIEIGF KVLKQNTSIRFEDTSLMPGASGTSDFDWAETITGYEVIQPDLLIVVEAEPLKDAASVLELDDKTKVK VGDIIATIKIENMKNFAGYQLNIK YDPTMLEAIELETGSAIAKRTWPVTGGTVLQSDNYGKTTAVAN DVGAGIINF AEAYSNLTKYRETGVAEETGIIGKIGFRVLKAGSTAIRFEDTTAMPGAIEGTYMFDWY ENIKGYSVVQPGEIVRSEGEPEGEEPTTEPVPTETSVDPTPTVTEEPVPSLPDSYVIMELDKTKVKV GDIITATIKIENMKNFAGYQLNIK YDPTMLEAIELETGSAIAKRTWPVTGGTVLQSDNYGKTTAVAN VGAGIINF AEAYSNLTKYRETGVAEETGIIGKIGFRVLKAGSTAIRFEDTTAMPGAIEGTYMFDWYGE NIKGYSVVQPGEIVVGPGEPEGEEPTTEPVPTETSVDPTPTVTEEPVPSLPDSYVIMELDKTKVKV GDIITATIKIENMKNFAGYQLNIK YDPTMLEAIELETGSAIAKRTWPVTGGTVLQSDNYGKTTAVAN VGAGIINF AEAYSNLTKYRETGVAEETGIIGKIGFRVLKAGSTAIRFEDTTAMPGAIEGTYMFDWYGENI KGYSVVQPGEIVAEGEPEGEEPTTEPVPTETSADPTPTVTEEPVPSLPDSYVIMELDKTKVKVGDIIA TIKIENMKNFAGYQLNIK YDPTMLEAIELETGSAIAKRTWPVTGGTVLQSDNYGKTTAVAN NFAEAYSNLTKYRETGVAEETGIIGKIGFRVLKAGSTAIRFEDTTAMPGAIEGTYMFDWYGENIKGYS VVQPGEIVAGPEGEPEGEEPTTEPVPTETPVDPTPTVTEEPVPSLPDSYVIMELDKTKVKVGDIIA KIENMKNFAGYQLNIK YDPTMLEAIELETGSAIAKRTWPVTGGTVLQSDNYGKTTAVAN VGAGIINF AEAYSNLTKYRETGVAEETGIIGKIGFRVLKAGSTAIRFEDTTAMPGAIEGTYMFDWYGENI KGYSVVQPGEIVARSEGEPEGEEPTTEPVPTETPVDPTPTVTEEPVPSLPDSYVIMELDKTKVKV KEGDIATIRVNNIK NLAGYQIGIKYDPK VLEAFNIETGDPIDEGTWPA VGGTILKNRDYLP TGVAINNVS KGLNFAAYVY FDDYREEGKSED TGIIGNIGFRVLKAEEDTTRIFEELSMPSGIDGTYMLD WYLNRRDYSYVVIQPAPIKA ASACGAWPSRVSKGEELFTGVVPIVLDGDVNGHKFVSSEGEEDATYGLKTLKFICTTGKLPVPW PTLVTLTYGVQCFSRYPDHMKQHDFK SAMPEGYVQERTIFFKDDGNYKTRAEVVKFEGD TLVNRIE LKIGDFKEDGNILGHKLEYNYNHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDG PVLLPDNHYLSTQSALS KDPNEKRDMVLEFVTAAGITLGMDELKYSADYLNRRDYSYVVIQPAPIKA TTTTDLTSINTSAYSTGSISTVETGNRTTSEVISHVVTSTKLSPTATTSLTIAQTSIYSTDSNITVGTDIH TTSEVISDVETISRETASTVVAAPTSTTGWGTAMNTYISQFTSSSFATINSTPIISSAVFETSDASIVNVH TENITNTAAVPSEPTFVNA TRNSLNSFCSSKQPSSPSSYTSSPLVSSLVSKTLLSTSTPTSPVPTNTYIK</p>

	TKNTGYFEHTALTTSSVGLNSFSETAVSSQGTKIDTFLVSSLIAYPSSASGSQLSGIQNFTSTSLMISTY EGKASIFFSAELGSHIFLLSYLLF
<i>Ta</i> LPMO-T (40 kDa)	PRFSKIIATAGVLASASLVAGHGfVQNIVIDGKKYYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVD GTGYQTPDIICHRGAKPGALTAPVSPGGTVELQWTPWPDSHHGPVINYLA PCNGDCSTVDKTQLEFF KIAESGLINDNPPGIWASDNLIAANNSWTVTIPTTIAPGNYVLRHEIHALHSAQNQDGAQNYQCINL QVTGGGSDNPAGTLGTALYHDTDPGILINIYQKLSYIIPGPPLYTGTSRPSGSAPRCTLQGGGDDGGS GGPQTYKVPGTPSTKLYGDVNDGKVNSTDAVALKRYVLRSGISINTDNADLNEDGRVNSTDLGILK RYILKEIDTLPYKNGSENLYFQGHHHHHHHH
<i>Mt</i> CDH-T (101 kDa)	PRRTSSRLIGALAAALLPSALAQNNAPVTFDTPDPSGITFNTWGLAEDSPQTKGGFTFVVALPSDALTT DAKEFIGYLKCARNDESGWCGVSLGGPMTNSLLIAAWPHEDTVYTSLRFATGYAMPDVYQGD AEIT QVSSSVNSTHFLSIFRCENCLQWSQSGATGGASTSNGVLVLGWVQAFADPGNPTCPDQITLQHDNG MGIWGAQLNSDAASPSYTEWAAQATKTVTGDGCGPTETSvVGVVPVPTGVSFYDVIYVGGGAGGIPAA DKLSEAGKSVLLIEKGFASANTGGTLGPEWLEGHDLTRFDVPGLCNQIWVDSKGIACEDTDQMAGC VLGGGTAVNAGLWFKPYSLDWDYLFPSGWKYKDVQPAINRALSRIPTDAPSTDGKRY YQQGFVDL SKGLAGGGWTSVTANNAPDKKNTFESHAPFMFAGGERNGPLGT YFQTAKKRNSFKLWLNTSVKRV RQGGHITGVEVEPRDGGYQGI VPVTKVTGRVILSAGTFGS AKILLRSGIGPNDQLQVVAASEKDGP MISNSSWINLPVGYNLDDHLNTDVISHPDVVFYDFYEA WDNPIQSDKDSYLSRKYGILAQAA PNIG MFWEIKGADGIVRQLQW TARVEGSLGAPNGKMTMSOYLGRGATS RGRMTITPSLT TVVSDVPYL KDPNDKEAVIQGIINLQNALKNVANLWLFPNSTITPRQYVDSMVVSPSNRRSNHWMGTNKIGTDDG RKGGSAVVDLNTKYVGTDNLFVIDASIFPGVPTTNPSTSYIVTASEHASARILALPDLTPVPKYGQCGG REWSGSFVCADGSTCQMNEWYSQCLTSRPSGSAPRCTLQGGGDDGSGGPQTYKVPGTPSTKLYG DVNDGKVNSTDAVALKRYVLRSGISINTDNADLNEDGRVNSTDLGILKRYILKEIDTLPYKNGSEN LYFQGHHHHHHHH
<i>Tr</i> EGIII-T (54 kDa)	PRQQTWVGQCGGIGWSGPTNCAPGSACSTLNPYYAQCPGATTITSTRPPSGPTTTTTRATSTSSSTPPT SSGVRFAGVSIAGDFGCTTDGTCVTSKYVYPLKNFTGSNNYPDGIGQM QHFVNEDGMTIFRLPVGW QYLVNNNLGGNLDSTSISKYDQLVQGCLSLGAYCIVDIHNYARWNGGIGQGGPTNAQFTSLWSQLA SKYASQSRVWFGIMNEPHD VNINTWAATVQEVVTAIRNAGATSQFISLPGNDWQSAGAFISDGSAAA LSQVTNPDGSTTNLIFDVHKYLDSDNSGTHAECTTNNIDGAFSPLATWLRQNNRQAILTETGGGNVQ SCIQDMCQQIYLNQNSDVYLG YVVGWAGSFDSTYVLTETPTGSGNSWTDTSLVSSCLARKTSGGG DDGGSGGPQGTTYKVPGTPSTKLYGDVNDGKVNSTDAVALKRYVLRSGISINTDNADLNEDGRVN STDLGILKRYILKEIDTLPYKNELENLYFQGHHHHHHHH
CBHII-T (58 kDa)	PRQQLWVGQCGGQGYSGATSCVAGATCATVNEYAQCPTAAGTSSATTLKTTTSSTTAAVTTTTT QSPTGSASPTTASASGNPFSGYQLVYNPYYSSEVASLAIPSLTGLSSLQAAAATAAKVPSFVWLDT AAKVPTMGDYLADIQSQNAAGANPPIAGQFVYVYDL PDRDCAALASNGEYSIADNGVEHYKSYIDSIR EILVQYSDVHTLLVIEPDSL ANLVTNLNVAKCANAESA YLECTNYALTQLNLPNVAMYLDAGHAGW LGWPANQQAADLFASVYKNASSPAAVRGLATNV ANYNAWTISSCPSYTOGNSVCDEQQYINAIAP LLQAQGFDAHFIVDTGRNGKQPTGQQA WGDWCNVINTGFGERP TTDGDALVDAFVWVKPGGESD GTSDDSA TRYDAHCGYSDALQPAEAGTW FQAYFVQLLTNANPAFTSGGGDDGSGGPQGTTYKVP GTPSTKLYGDVNDGKVNSTDAVALKRYVLRSGISINTDNADLNEDGRVNSTDLGILKRYILKEIDT LPYKNELENLYFQGHHHHHHHH
<i>Npa</i> BGS-T (96 kDa)	PRITWEEADAKAREWCADLTNEEKISIITGRENMTGVCVGSIDPIERKGFKGLCLQDGPAGVRFKGT ATSWQASINSAATFDRILLRKVGEAQGNEFYQRGINFALAPSVGIQRAPASGRIWESYGEDPFYV GEC GTEVVKGIQSQGVIA TSKHFGNDQENNRGASTSNIP EQALWEVYLA PFYRLVND AETNAIMSSYNA VNGTYTSENKRLT DILKDKMGFQGMVMSDWWGLYRIDSFGAGLDMNMPGGKYWGPDYVGD SF WGEHIQEYIDQGIFTQERLDDAALRVIRALFKAGQ MENFPEVNL YVDTLTEENIALNRKVGADSNVL LKNDESVLPIKGVKIAVIGKDSMPANFCEDMRCADGTLALGWGSGTTDFKYVIDPLSAITERAKKD NIEVVSSGEDNSEAGA EVAKDADLAIVFVQADS GEEYITVEGNAGDRLNLDLWHGGNELIDAVASV NKNTIVVIHAPGPVNPFLDKVKGVV FAGMPQGESGHAIADVLF GDVNPSGHLPYTWAPREDYPTD VKYEPEYPDGGEKMTVYDYN EGLFVGYRWFDKQGI EPTFAFGYGLSYTTFEYSNLEAVMEEDGLHV TLTVTNTGDVAGAAVPMIFLSFPDIVKDYPSRLFKGFDKVMLEAGESKQVNIIVDNHDL SYYDVDAE KFVKPEEGEYTVFAGSNARDLPLKTTVLANGE CNSDEEVSGNVENDEDSVDEVDAETENAEDSADE VDSEVDAENAEDSVDETALKKRAYKLYTSRPSGSAPRCTLQGGGDDGSGGPQTYKVPGTPSTKLY GDVNDGKVNSTDAVALKRYVLRSGISINTDNADLNEDGRVNSTDLGILKRYILKEIDTLPYKNGSEN LYFQGHHHHHHHH

Table S6: Detailed comparison of fermentation parameters of previous studies and this study.

Host name	Genes	Number of enzyme binding sites	Initial OD (A ₆₀₀)	Ethanol (g/l)			*Fermentation time (h)	Fermentation temperature (°C)	Reference
				CMC	PASC	Avicel			
<i>Saccharomyces cerevisiae</i> BY4742	<i>Ct</i> Exo, <i>Tr</i> CBHII, <i>Ta</i> BGL1	3	50	-	1.87	-	48	30	Tsai et al., 2009 (7)
<i>S. cerevisiae</i> EBY100	<i>Tr</i> EGII, CBHII, <i>Aa</i> BGI1	3	50	-	1.80	-	70	30	Wen et al., 2010 (8)
<i>S. cerevisiae</i> BY4742	<i>Ct</i> CelA, <i>Tr</i> CBHII, <i>Ta</i> BGL1	3	0.8	-	1.25	-	144	30	Goyal et al., 2011 (9)
<i>S. cerevisiae</i> EBY100	<i>Cc</i> CelccA, celCCE, <i>Ccel</i> 2454	12	50	1.00	1.09	1.41	96	30	Fan et al., 2012 (10)
<i>S. cerevisiae</i> EBY100	<i>Cc</i> CelG, <i>Bglf</i>	4	50	-	1.90	-	72	30	Tsai et al., 2013 (11)
<i>S. cerevisiae</i> EBY100 <i>S. cerevisiae</i> HZ848	CBH2, EG2, BGL1, LPMO, and CDH	5	50	-	2.70	1.5	96	30	Liang et al., 2014 (12)
<i>S. cerevisiae</i> EBY100	CBH, EG, BGL and <i>Cdt1</i>	8	0.1	3.26	1.09	-	60	30	Fan et al., 2016 (13)
<i>S. cerevisiae</i>	CBHI, CBHII, EG, BGL	Tethered	150 g wet cells/L	-	6.7	1.4	96	37	Liu et al., 2016 (14)
<i>Kluyveromyces marxianus</i> 4G5	<i>Tr</i> EgIII, CBHII, <i>Npa</i> BGS, <i>Ta</i> LPMO, <i>Mt</i> CDH	63	20	-	8.89	3.09	144/PASC 120/avicel	37	This study

*Fermentation time (h): When the highest ethanol production was observed.

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