Supplementary Materials: Enhancing Enzymatic Properties of Endoglucanase I Enzyme from *Trichoderma Reesei* via Swapping from Cellobiohydrolase I Enzyme

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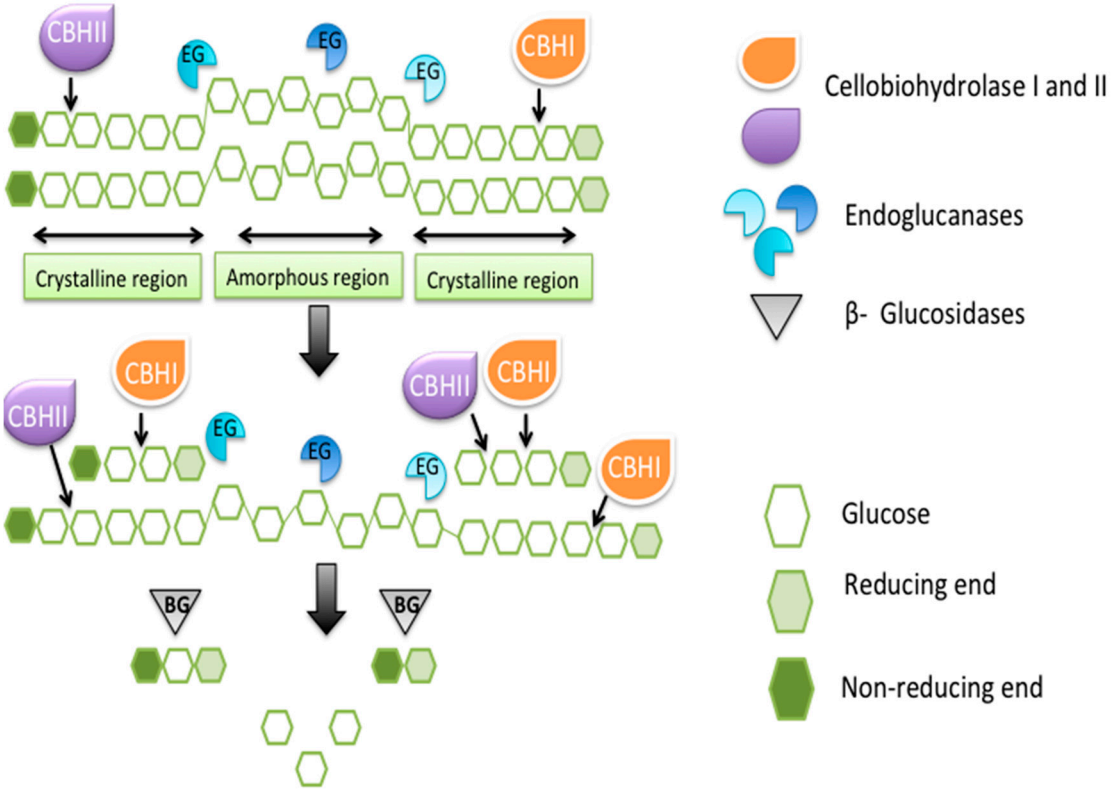

Figure S1. The depiction of cellobiohydrolase and endoglucanase mode of action.

The difference in CBHI's and EGI's mode of action is described. Specifically, endoglucanase enzymes are attacked to amorphous region of cellulose chain and hydrolyzed the accessible intramolecular β–1,4-glucosidic bonds through generating new ends while cellobiohydrolase enzymes are attacked to crystalline region of cellulose chain.

a. TM-align results

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(":" denotes aligned residue pairs of d < 5.0 A, "." denotes other aligned residues)
                                                *                                 *
EG1  QPGTSTPEVHPKLTTYKCTKSGGCVAQDTSVVLDWNYRWMH D-ANYNSCTVNGGVNTTLCPDEATCGKNCFIEGVDYAA-SG VTTSGSSL
     ::::::::::::::::::::::.:::::::::::::::::: : ::::::::::::::::::..:::::::::::::::: :: ::::::::
Cbh1 SACTLQSETHPPLTWQKCSSGGTCTQQTGSVVIDANWRWTH ATNSSTNCYDGNTWSSTLCPDNETCAKNCCLDGAAYASTYG VTTSGNSL

EG1  TMNQYMPSSSGGYSSVSPRLYLLDSDGEYVMLKLNGQELSFDVDLSALPCGENGSLYLSQMDENGGA--NQYNTAGANYGSGYCDAQCPV
     :::::::::..   ::::::::::::.:::::::::::::::::::::::::::::::::::::::  :::::::::::::::::::::
Cbh1 SIGFVTQSAQ---KNVGARLYLMASDTTYQEFTLLGNEFSFDVDVSQLPCGLNGALYFVSMDADGGVSKYPTNTAGAKYGTGYCDSQCPR
                          **
EG1  -QTWRNGTLN-TS-------------HQGFCCNEMDILEGNSRANALTPHSCTA----------------------TACDSAGCGFNPYGS
      ::::::::: ..             ::::::::::::::::::::::::::::                      .:::::::::::::::
Cbh1 DLKFINGQANVEGWEPSSNNANTGIGGHGSCCSEMDIWEANSISEALTPHPCTTVGQEICEGDGCGGTYSDNRYGGTCDPDGCDWNPYRL

EG1  GYKSYYGPG--DTVDTSKTFTIITQFNTDNGSPSGNLVSITRKYQQNGVDIPSAQP-----GGDTISS--CP---------SASAYGGLAT
     :::::::::  ::::::::::::::::::       : ::::::::::::::::.     ..::::::  ::         ::::::::::
Cbh1 GNTSFYGPGSSFTLDTTKKLTVVTQFETS-------G-AINRYYVQNGVTFQQPNAELGSYSGNELNDDYCTAEEAEFGGSSFSDKGGLTQ

EG1  MGKALSSGMVLVFSIWNDNSQYMNWLDSG------------NAGPCSSTEGNPSNILANNPNTHVVFSNIRWGDIGSTT----
     ::::::::::::::::::::::::::::::            ::::::::::::::::::::::::::::::::::::::::
Cbh1 FKKATSGGMVLVMSLWDDYYANMLWLDSTYPTNETSSTPGAVRGSCSTSSGVPAQVESQSPNAKVTFSNIKFGPIGSTGNPSG
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b. The structural representation of alignment results

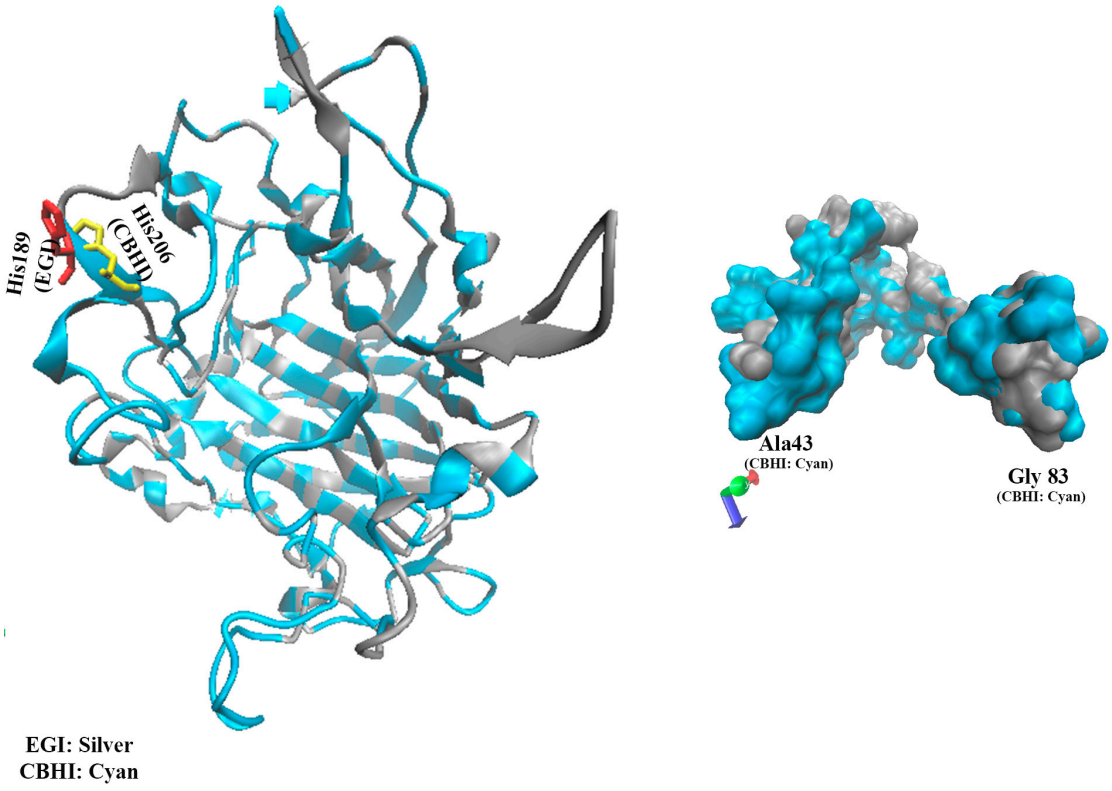

Figure S2. The structural alignment results between EGI and CBHI enzymes (a) TM-alignment results between enzymes are displayed. ':' donates aligned residue pairs whose distances are smaller than 5 Å. The aligned region, Ala43-Gly83 (CBHI numbering) is indicated with *. The structural break happened around His189 (EGI numbering) is indicated with **. (b) The structural presentation of alignment results are provided in 'cartoon' format for overall, and 'surf' format just for swapped region. His206 in CBHI and His189 in EGI are drawn in 'licorice' format as yellow and red, respectively. In 'surf' presentation, the end points of swapped domain (Ala43 and Gly 83 as CBHI numbering) are indicated.

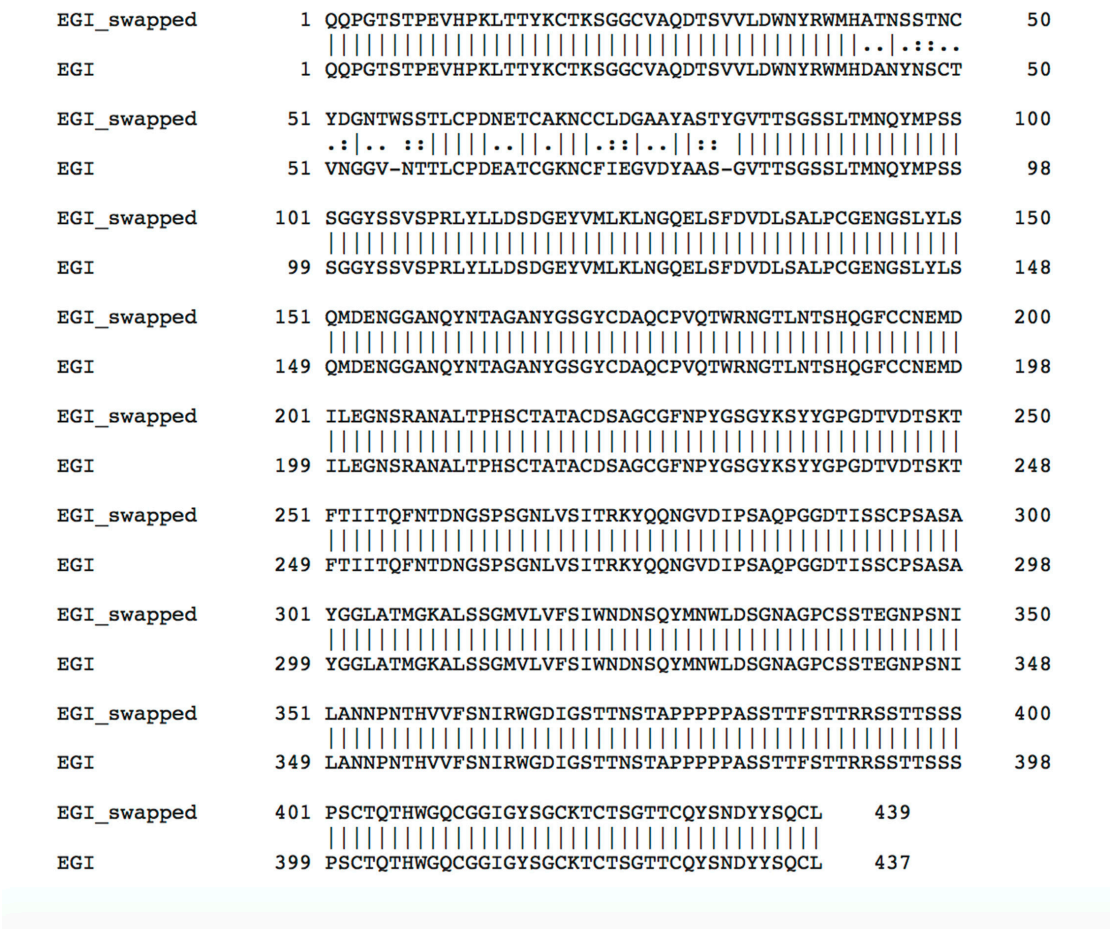

Figure S3. The multiple protein alignment results between EGI_swapped and EGI.

Here, the full conservation, acceptable changes and dramatic changes are indicated with vertical' line, ':' and '.', respectively.

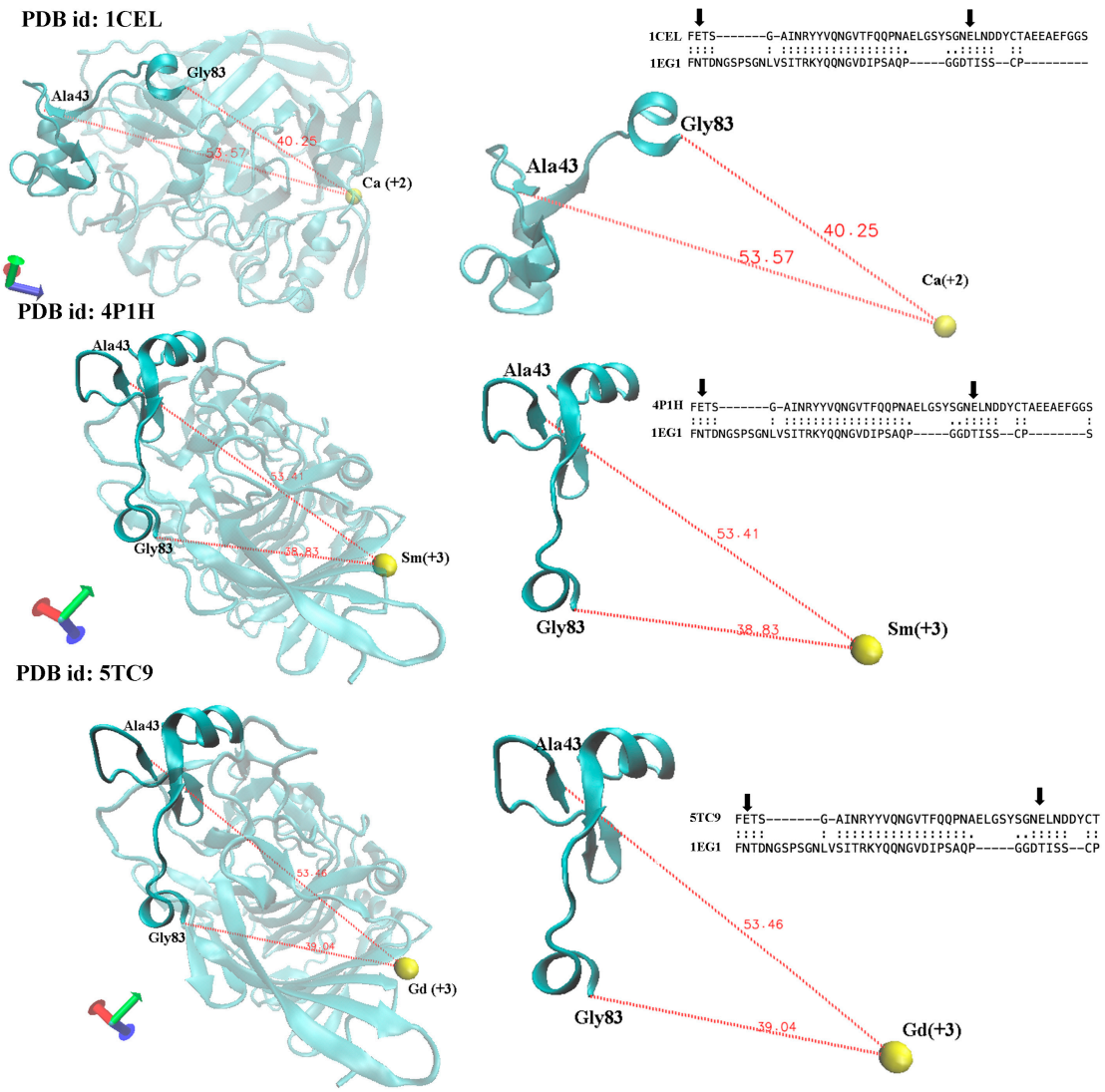

Figure S4. The crystal structures of cellobiohydrolase I enzymes. These cellobiohydrolase I enzymes have displayed more than 95% sequence similarity to CBHI (pdb id: 1DY4). These structures are crystallized with metal ions different than Co^{2+} ion. On the left side, the crystal structures are displayed with metal ions. Structures and metal ions are drawn in 'cartoon' and 'VDW' format, respectively, with Visual Molecular dynamics. The corresponding parts of our swapped region, Ala43-Gly83, in cellobiohydrolase enzymes are displayed by indicating the distances of Ala43 and Gly83 to metal ions. On the right side, the structural alignment results of cellobiohydrolase enzymes, pdb id: 1CEL, 4P1H and 5TC9, with EGI enzyme (pdb id: 1EG1) are displayed by indicating Glu residues involved in metal ions coordination.

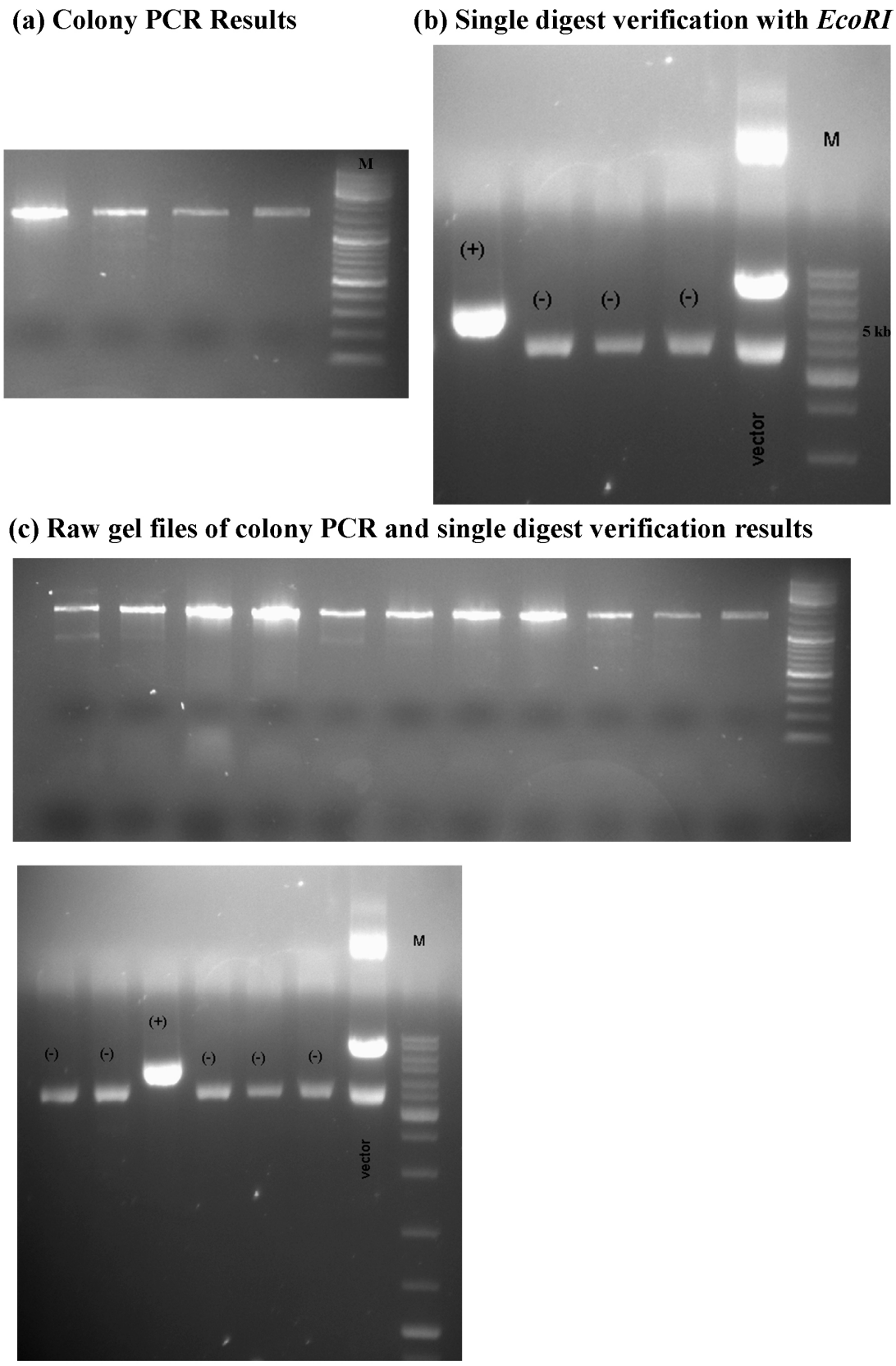

Figure S5. The cloning results of EGI_swapped; (**a**) the colony PCR results and (**b**) the double verification of isolated plasmid with *EcoR* I, and (**c**) Raw files of colony PCR results and single digest verification with *EcoR* I. After selection of positive clones through colony PCR, the isolated plasmids (last four ones) were double checked with

single *EcoR* I digestion as displayed in (b). The colony PCR's products and linearized plasmid were visualized in 1.2% w/v Agarose gel by ethidium bromide staining. M indicates a GeneRuler Ladder Mix (Fermentas).

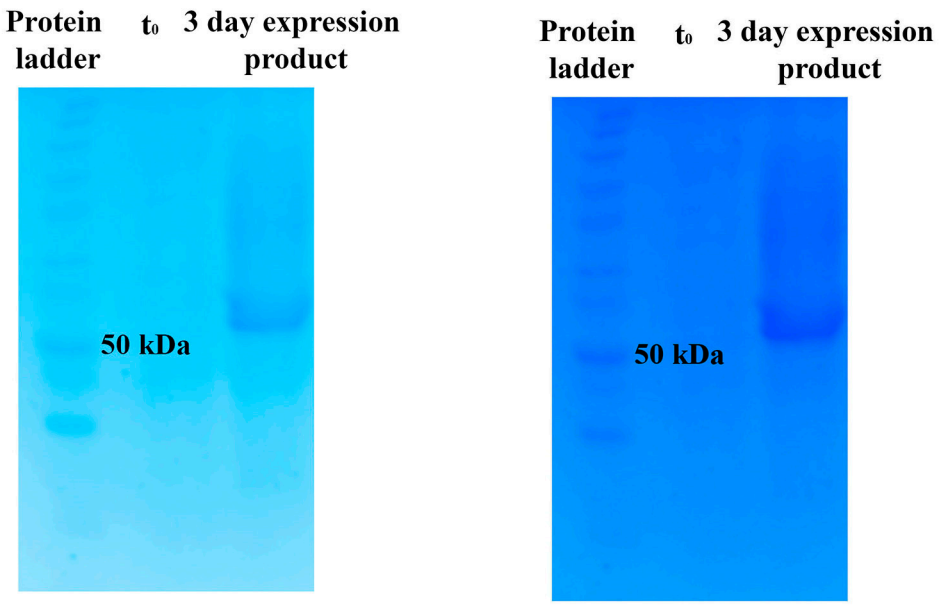

Figure S6. SDS-PAGE results of EGI_swapped.

In SDS-PAGE, only EGI_swapped samples at t_0 and t_3 were only loaded with unstained protein ladder (Fermentas) as a marker. SDS-PAGE was prepared as % (w/v) stacking and 12% (w/v) separation gel. Prior to loading, they were boiled at 95°C for 5 min. After staining of gel with Coomassie Brillant Blue R-250, this raw picture was captured with ChemiDoc Imaging Systems (Bio-Rad) as full length by focusing only the loaded wells with white-plate, where SDS-staining visualization was chosen.

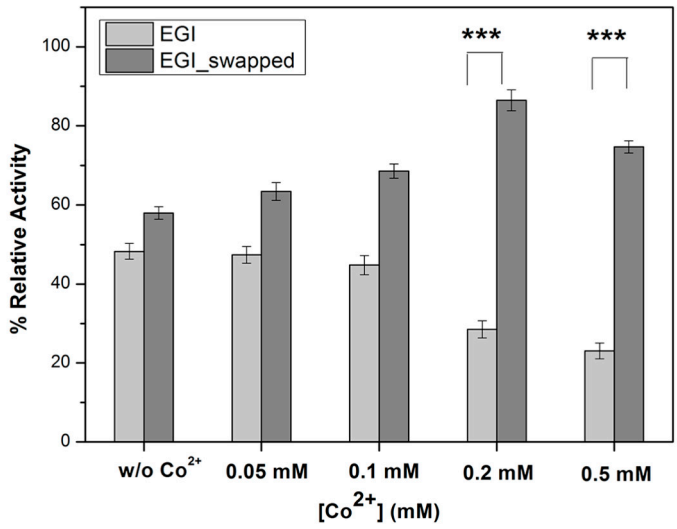

Figure S7. The effects of Co2+-ion pre-incubation with CMC prior to hydrolysis reaction on their enzymatic activity profiles.

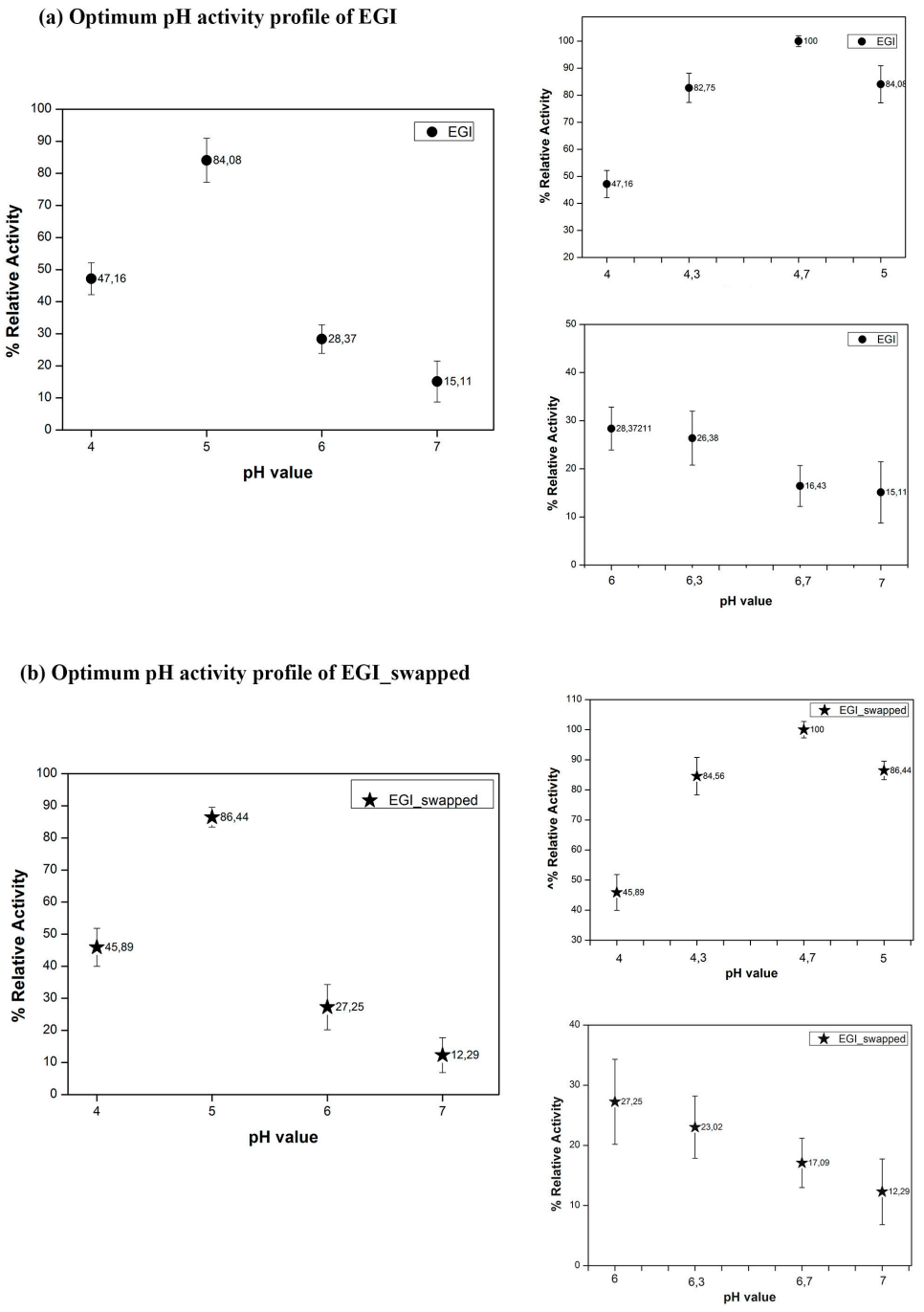

Figure S8. The construction of pH profiles for native and domain swapped enzymes. Before performing activity and thermal stability assays, the impact of domain swapping from CBHI to EGI enzyme on optimum pH value was revealed. For pH 4-5 ranges and pH 6-7 ranges, 50 mM sodium acetate and 50 mM potassium phosphate buffers were used, respectively. For each native and domain swapped enzymes, their maximum enzymatic activities were set to 100% to calculate the rest. The relative activities of EGI (a) and EGI_swapped (b) are displayed as 'black circle' and 'black star' with normalized standard deviations below 10%. Data are expressed as mean ± SD, n=3.

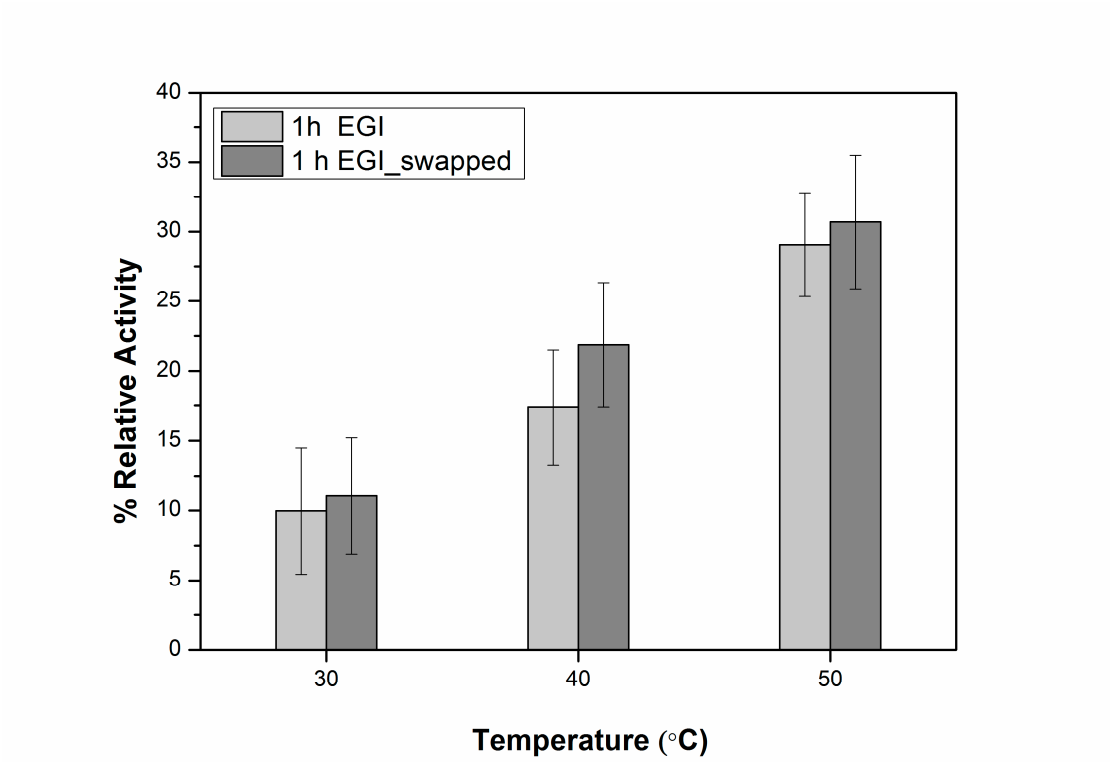

Figure S9. The enzyme activity profiles of EGI and EGI_swapped enzymes toward CMC for 1 hour at 30-50˚C(/10˚C).

Among all data set, the highest enzymatic activity value, corresponding to EGI_swapped's activity at 60˚C for 6 h, was set to 100% to calculate the rest of relative activities belonging to EGI and EGI_swapped enzymes. The results are displayed with normalized standard deviations, below 10%.

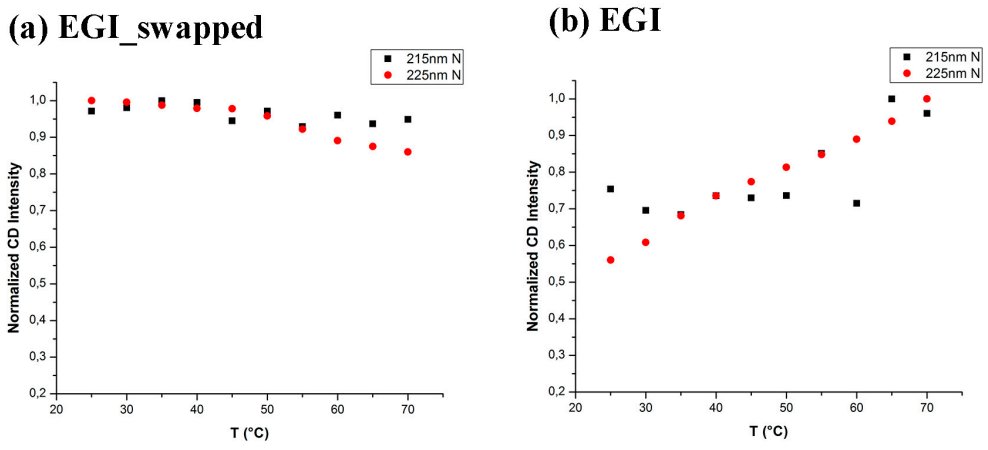

Figure S10. The temperature path of EGI_swapped and EGI enzymes.

Table S1.

Name	Length of protein (aa)	Length of nucleotide sequence (bp)	Predicted molecular weight (kDa)	Predicted molecular PI
EGI_swapped	439	1395	~46,3 kDa	4.70
EGI	437	1527	46 kDa	4.66