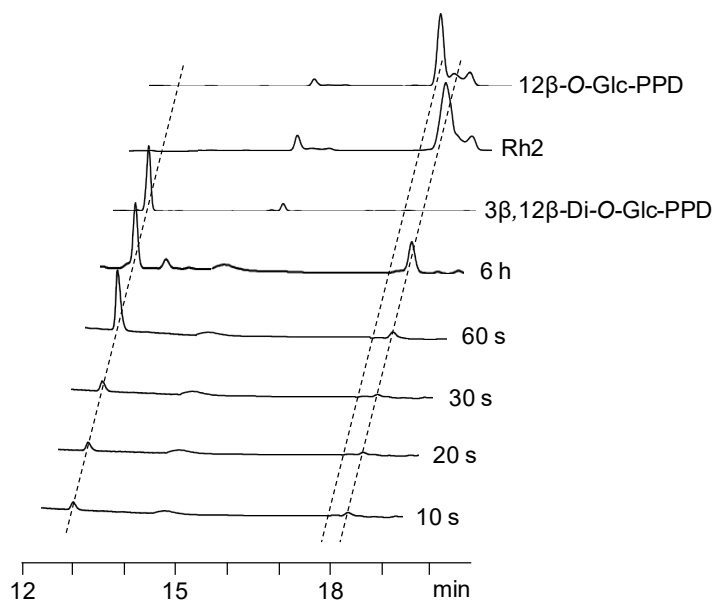
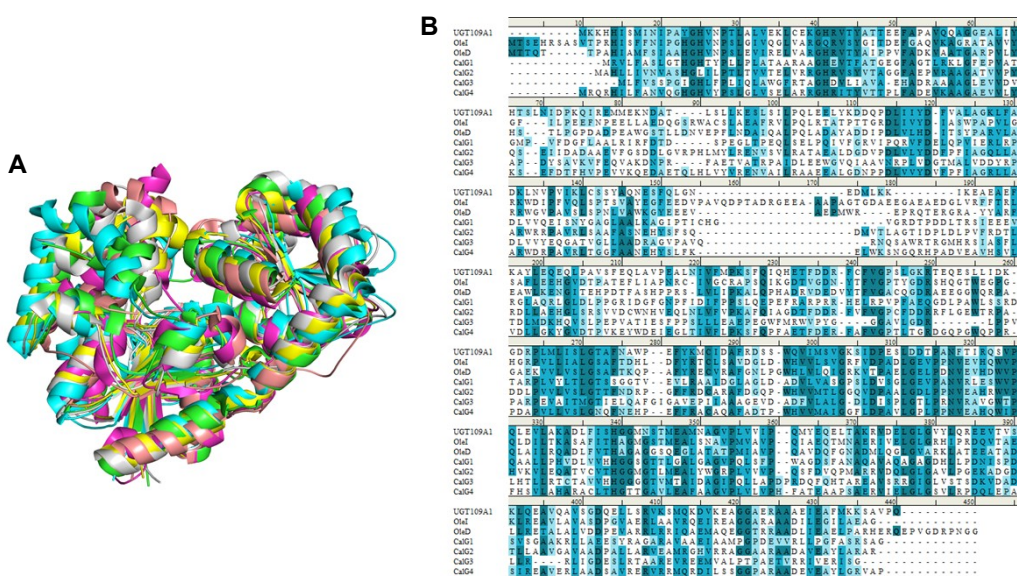


## Supplementary Information

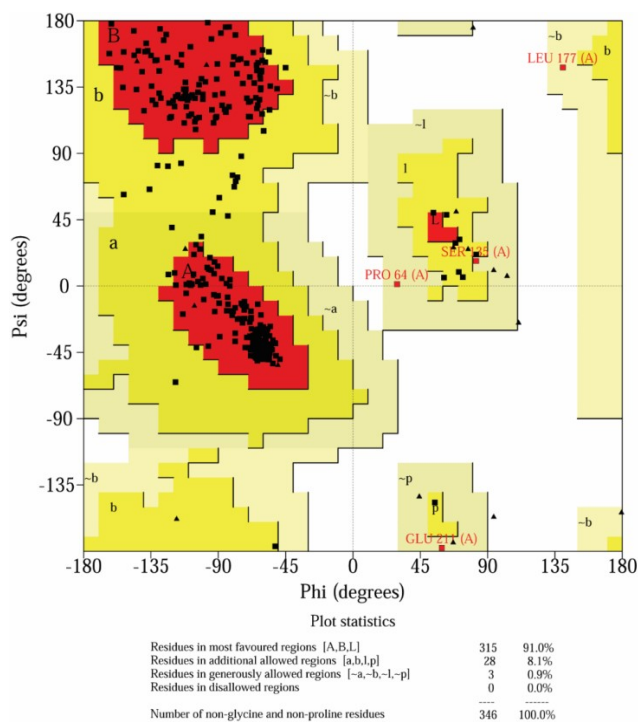
**Supplementary Fig. 1** HPLC analysis of the reaction products of UGT109A1 towards PPD in different reaction time.



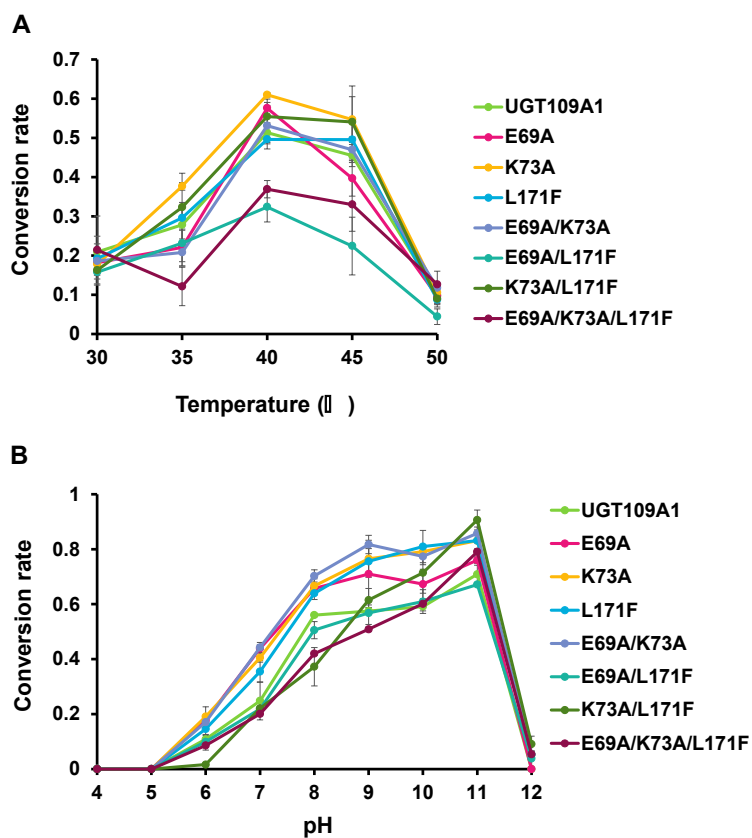
**Supplementary Fig. 2** Structure and sequence alignments of GT1 family members. (A) Structure alignment. OleI (2IYA), OleD (2IYF), CalG1 (30TH), CalG2 (3RSC), CalG3 (30TI) and CalG4 (3IA7) are colored in green, cyan, magenta, yellow, salmon, and gray, respectively. (B) Sequence alignment of UGT109A1 with other GT1 members. The blue shading indicates the amino acid similarity (matching degree above 70%). Dark blue represents identity, medium blue represents strong similarity, light blue represents weak similarity.



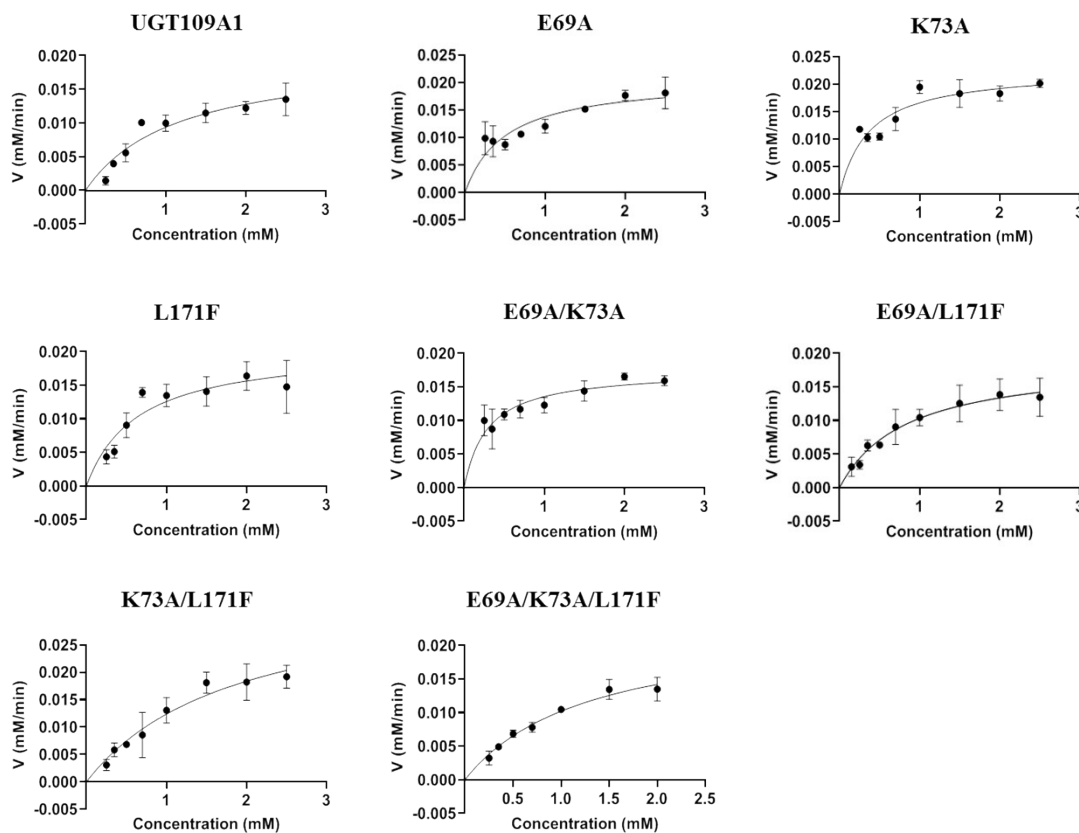
**Supplementary Fig. 3** Evaluation of the predicted structure of UGT109A1 by Ramachandran plot.



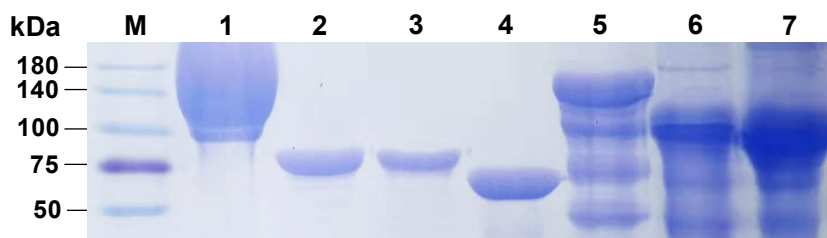
**Supplementary Fig. 4** Temperature (A) and pH (B) profiles of UGT109A1 and its mutants.



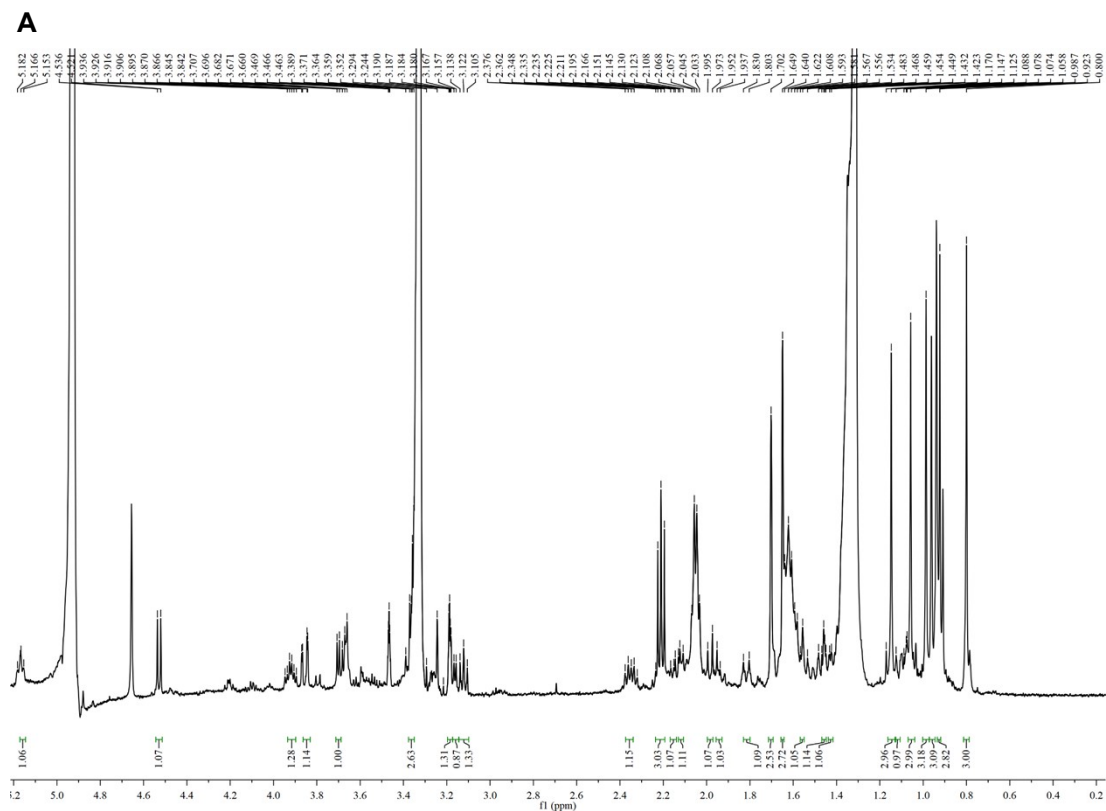
**Supplementary Fig. 5** The kinetic curves of WT UGT109A1 and mutants towards PPD.



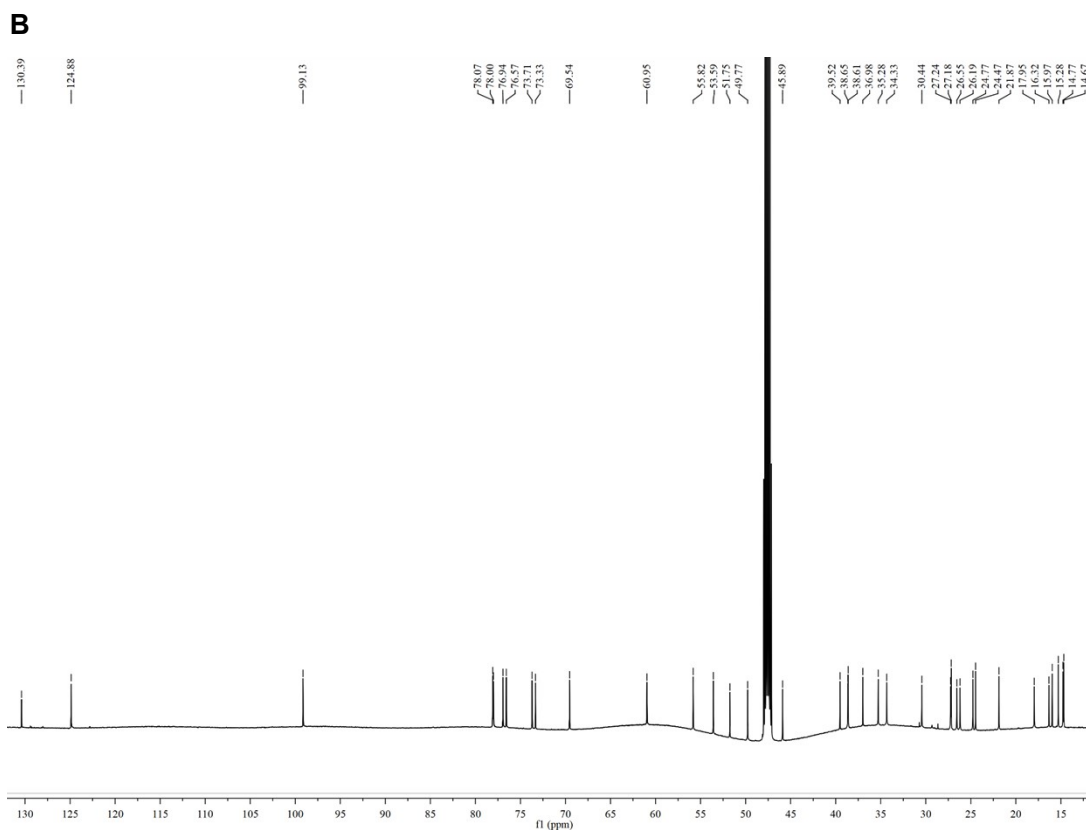
**Supplementary Fig. 6** SDS-PAGE analysis of purified recombinant  $\beta$ -glycosidases. M: protein marker; 1: purified recombinant P1-2 with molecular weight of 97 kDa; 2: purified recombinant Xyl3 with molecular weight of 84 kDa; 3: purified recombinant Bgy2 with molecular weight of 83 kDa; 4: purified recombinant MT619 with molecular weight of 68 kDa; 5: purified recombinant EGH1 with molecular weight of 138 kDa; 6: purified recombinant EXG1 with molecular weight of 99 kDa; 7: purified recombinant SPR1 with molecular weight of 100 kDa.



**Supplementary Fig. 7** The  $^1\text{H}$ -NMR (A) and  $^{13}\text{C}$ -NMR (B) spectra of  $12\beta\text{-O-Glc-PPD}$  produced from  $3\beta,12\beta\text{-Di-O-Glc-PPD}$  by *in vitro* reaction with  $\beta$ -glycosidases.

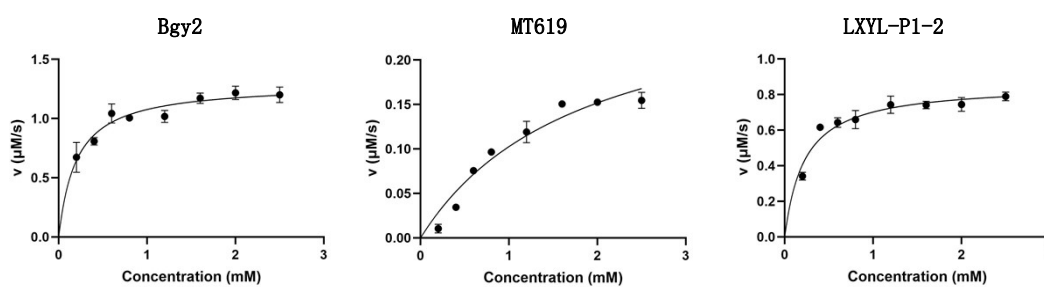


$^1\text{H}$ -NMR (500MHz, in Methanol- $d_4$ )  $\delta$  5.16 (1H, t,  $J = 14\text{Hz}$ , H-24), 4.52 (1H, d,  $J = 10.0\text{Hz}$ , H-1'), 3.16 (1H, dd,  $J = 5\text{Hz}, 5\text{Hz}$ , H-3), 3.12 (1H, m, H-12), 1.70 (3H, s, H-27), 1.65 (3H, s, H-26), 1.15 (3H, s, H-21), 1.07 (3H, s, H-28), 1.05 (3H, s, H-29), 0.98 (3H, s, H-18), 0.92 (3H, s, H-30), 0.80 (3H, s, H-19).



$^{13}\text{C}$ -NMR (125 MHz, Methanol- $d_4$ )  $\delta$  131.39, (C-25), 124.88, (C-24), 76.94, (C-3), 78.07, (C-12), 73.71, (C-20), 55.82, (C-5), 53.59, (C-17), 51.75, (C-14), 49.77, (C-9), 45.89, (C-13), 39.52, (C-8), 38.65, (C-4), 38.61, (C-1), 36.98, (C-10), 35.28, (C-22), 34.33, (C-7), 30.44, (C-15), 27.24, (C-28), 27.18, (C-2), 26.55, (C-11), 26.19, (C-16), 24.77, (C-21), 24.47, (C-26), 21.87, (C-23), 17.95, (C-6), 16.32, (C-27), 15.97, (C-19), 15.28, (C-29), 14.77, (C-30), 14.67, (C-18), 99.13, (C-1'), 73.33, (C-2'), 76.57, (C-3'), 69.54, (C-4'), 78.00, (C-5'), 60.95, (C-6').

**Supplementary Fig. 8** The kinetic curves of Bgy2, MT619 and LXYL-P1-2 towards  $3\beta,12\beta$ -Di-*O*-Glc-PPD



**Supplementary Table 1** The HPLC conditions for analysis and preparation of products.

Gradient	Product
0 min, 20% ACN; 20 min, 85% ACN; 30 min, 100% ACN; 40 min, 100% ACN; 41 min, 20% ACN; 50 min, 20% ACN	PPD Rh2 3 $\beta$ ,12 $\beta$ -Di- <i>O</i> -Glc-PPD 12 $\beta$ - <i>O</i> -Glc-PPD