A novel β-glucuronidase from *Talaromyces pinophilus* Li-93 precisely hydrolyzes glycyrrhizin into glycyrrhetinic acid 3-*O*-mono-*β*-D-glucuronide

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TABLE S1 The specific activity and catalytic efficiency of the published GUSs on GL

Enzyme	specific activity ($\mu mol^{-1}\ min^{-1}\ mg^{-1}$)	k_{cat}/K_m ($mM^{-1}s^{-1}$)	Reference
TpGUS79A	11.97	11.14	This study
β-glucuronidase I	0.32	NA*	(1)
β-glucuronidase II	0.19	NA	(1)
PGUS-P	9.79E-3	6.40E-4	(2)
PGUS-E	8.62E-3	3.86E-3	(2)
AtGUS-E	NA	2.24	(3)

* Not available

TABLE S2 The specific activity and catalytic efficiency of the published GUSs on baicalin

Enzyme	Specific activity ($\mu mol^{-1}min^{-1}mg^{-1}$)	k_{cat}/K_m ($mM^{-1}s^{-1}$)	Reference
TpGUS79A	0.56	0.29	This study
LcGUS30	7.57	14.0	(4)
SvGUS	9.37	NA*	(5)

* Not available

References

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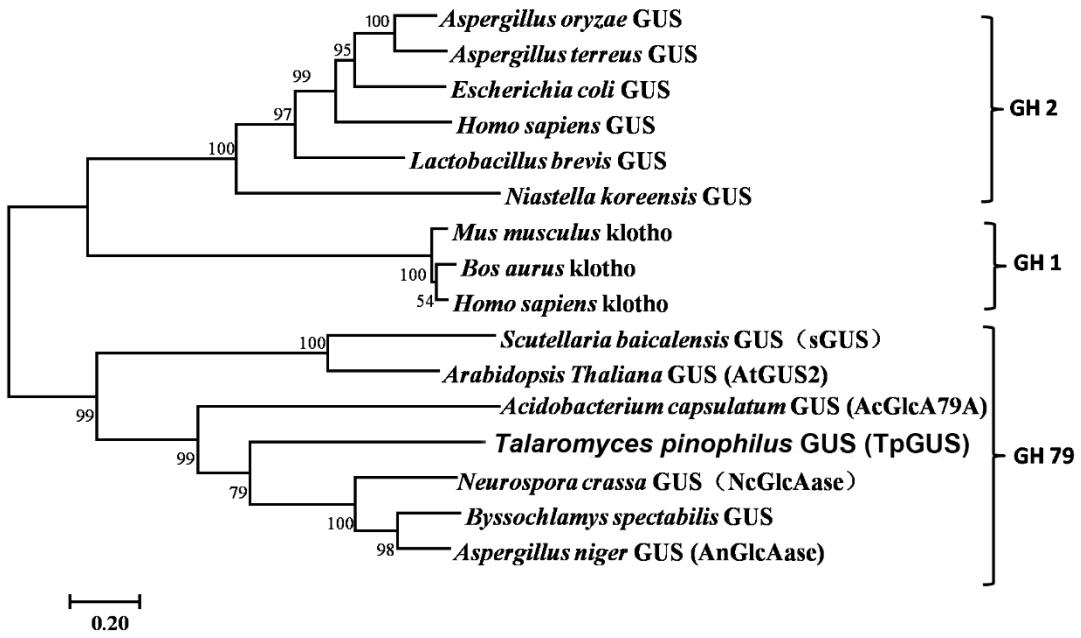

FIG S1 The phylogenetic analysis of TpG79A with other GUSs from GH1, GH2 and GH79 based on their amino acid sequences. The phylogenetic tree was constructed using MEGA 7 with a neighbor-joining method. On the phylogenic tree, the GH79 GUSs include enzymes from *S. baicalensis* (10/29(34%) identity, AB040072), *A. Thaliana* (26/111(23%) identity, NP_196400), *A, capsulatum* (114/459 (25%) identity, PDB: 3VNY_A), *N. crassa* (144/432(33%) identity, XP_964763), *B. spectabilis* (160/462 (35%) GAD97470), and *A. niger* (128/377(34%) identity, GAQ47476); The GH2 GUSs include enzymes from *A. oryzae* (ABU68712), *A. terreus* (AEP39213), *E. coli* (PDB: 3K46_A), *Homo sapiens* (PDB: 3HN3_A), *L. brevis* (ACU21612) and *N. koreensis* (AEW00660); The GH1 GUSs include enzymes from *M. musculus* (BAA23381), *B. aurus* (NP_001178124), *Homo sapiens* (BAA23382).

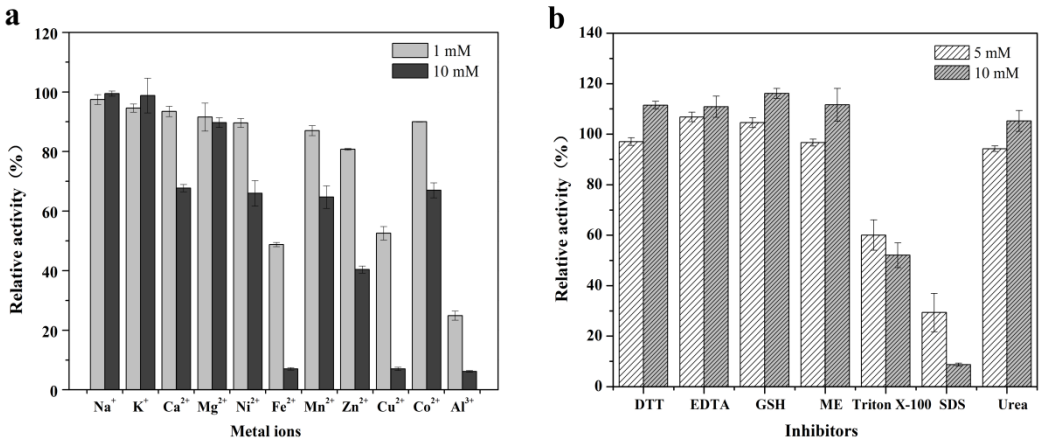

FIG S2 Effect of metal ions (a) and inhibitors (b) on TpGUS79A activity. The experiment was performed in triplicate measurements, and the errors stand for one standard deviation.

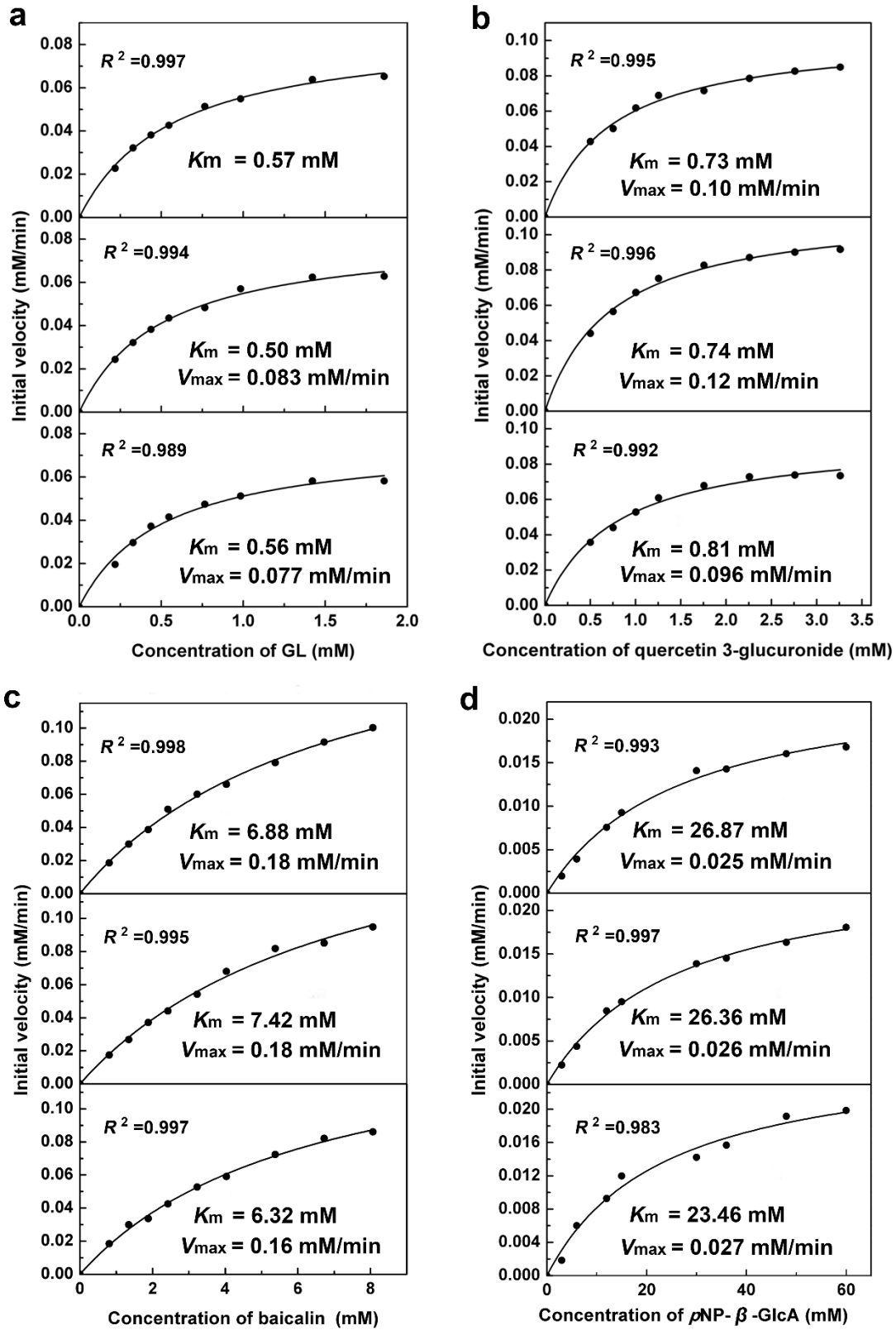

FIG S3 Michaelis-Menten plots of TpGUS79A hydrolyzing the different substrates. a: glycyrrhizin (GL), b: quercetin 3-glucuronide, c: baicalin, d: *p*NP-*β*-GlcA.

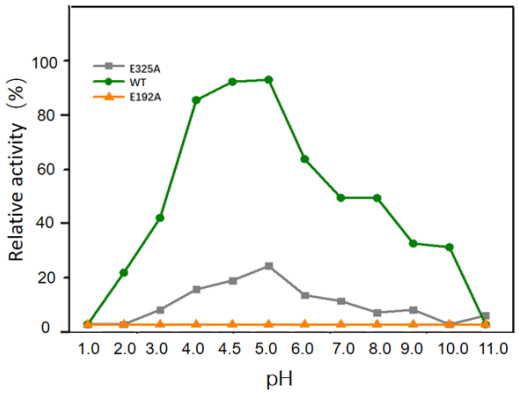

FIG S4 Activity rescue test of TpGUS79A mutants E192A and E325A.

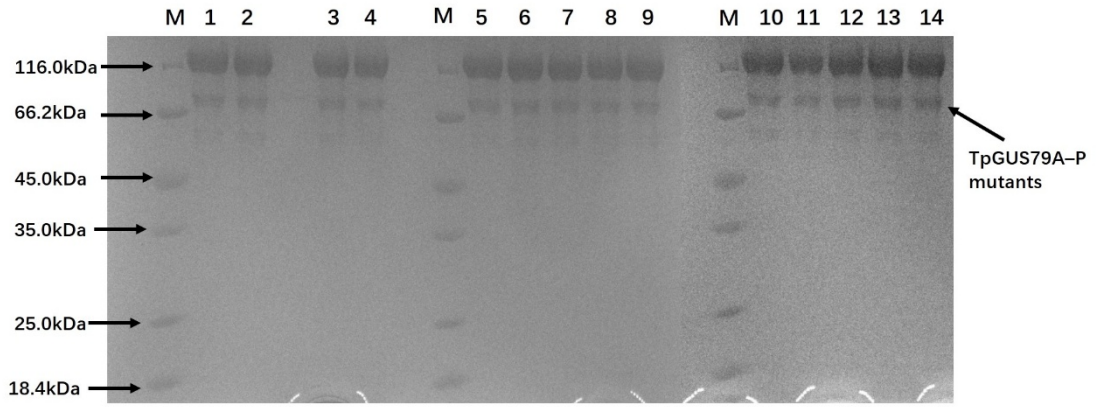

FIG S5 The SDS-PAGE of the purified TpGUS79A–P mutants. Lane M: marker, Lane 1: S330A, Lane 2: H365D, Lane 3: E192A, Lane 4: N191A, Lane 5: C331A, Lane 6: Y372K, Lane 7: C331D, Lane 8: E325A, Lane 9: Y372D, Lane 10: C331K, Lane 11: Y276A, Lane 12: S330R, Lane 13: S95A, Lane 14: Y372A.

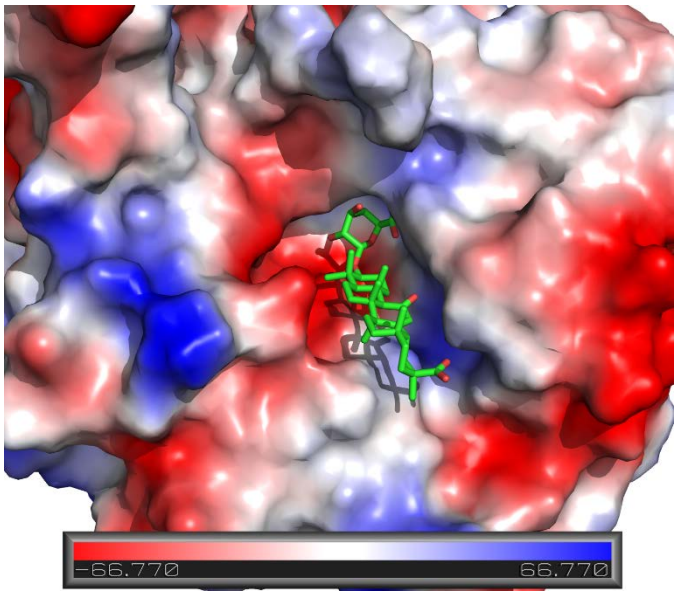

FIG S6 The electrostatic potential distribution of the active pocket of GH2 *β*-glucuronidase from *Aspergillus oryzae* Li-3. The green stick stands for the substrate glycyrrhetinic acid 3-*O*-mono-*β*-D-glucuronide. The image was reproduced from PDB 5C71.

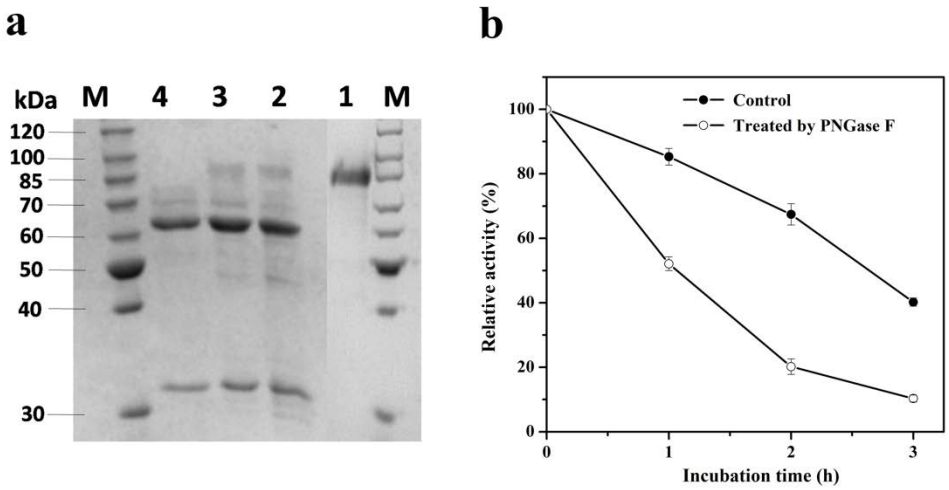

FIG S7 The effect of N-glycosylation on TpGUS79A activity (a) SDS-PAGE analysis of TpGUS79A treated with PNGase F in the non-denaturing reaction condition at different incubation time. Lane 1: 0 h, Lane 2: 1 h, Lane 3: 2 h, Lane 4: 3h. (b) The activity of TpGUS79A natively deglycosylated by PNGase F on GL. The activity for GL was assayed at 50 °C for 5 min in 50 mM NaAc-HAc buffer (pH 4.5). Data represent the mean ± one standard deviation of results from the experiments in triplicate.