Supporting Information

Engineering galactose oxidase for efficient cascade synthesis of L-guluronic acid from D-glucose

Qingxiu Wang[a,b,#], *Weisong Liu*[b,c,#], *Lijing Chang*[b], *Kuncheng Zhang*[b,c], *Yanbing Shen*[a], *Lingling Zhang*[b,c,*]

[a] College of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, P.R. China

[b] Key Laboratory of Engineering Biology for Low-carbon Manufacturing, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, China

[c] University of Chinese Academy of Sciences, Beijing 100049, P. R. China

[#] These authors contributed equally to this work

* Corresponding author: Lingling Zhang, zhangll@tib.cas.cn

Table of Contents

Supporting Tables

Table S1 Mutagenesis primers used in this work

Primer name	Primer sequence（5’-3’）
W290F-F	GTGGTAGTTTTAGCGGTGGTGTTTTTGAG
W290F-R	ACACCACCGCTTTTACTACCACCAATGG
R330K-F	CAGGGTCTGTATAAAAGCGATAATCATGCATGGC
R330K-R	TGATTATCGCTAAAATACAGACCCTGTTTATCTGCG
Q406T-F	ACCGGATTATACCGATAGTGATGCAACCACC
Q406T-R	TGCATCACTATCGGTATAATCCGGTGAACCACC
T406K-F	ACCGGATTATAAAGATAGTGATGCAACCACC
T406K-R	TGCATCACTATCTTTATAATCCGGTGAACCACC
T406R-F	ACCGGATTATCGTGATAGTGATGCAACCACC
T406R-R	TGCATCACTATCACGATAATCCGGTGAACCACC
F290NNK-F	GTGGTAGTNNKAGCGGTGGTGTTTTTGAG
F290NNK-R	ACACCACCGCTMNNACTACCACCAATGG
Y329NNK-F	AAACAGGGTCTGNNKAAAAGCGATAATCATG
Y329NNK-R	ATCGCTTTTMNNCAGACCCTGTTTATCTGC
K330NNK-F	AGGGTCTGTATNNKAGCGATAATCATGCATG
K330NNK-R	TGATTATCGCTMNNATACAGACCCTGTTTATC
Y405NNK-F	TTCACCGGATNNKCGTGATAGTGATGC
Y405NNK-R	ACTATCACGMNNATCCGGTGAACCACC
P463NNK-F	TCGTGGTATTNNKTTTGAAGATAGTACAC
P463NNK-R	TATCTTCAAAMNNAATACCACGACGCTGAC
F464NNK-F	TGGTATTCCGNNKGAAGATAGTACACCGG
F464NNK-R	TACTATCTTCMNNCGGAATACCACGACGCTGAC
H496NNK-F	TGCGTGCCTATNNKAGCATTAGCCTGC
H496NNK-R	CTAATGCTMNNATAGGCACGCACAATGC

Supporting Figures

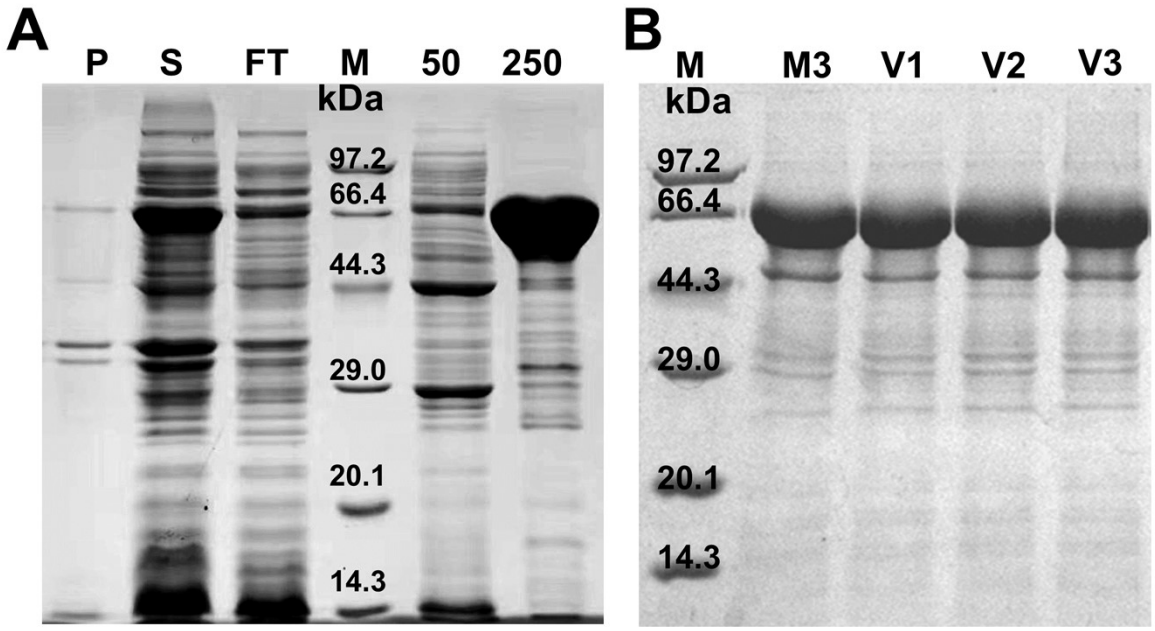

Figure S1. (A) GOase M3 purified by Ni-NTA column. P: Precipitation, S: Supernatant, FT: Flow through, M: Maker, 50: Eluent containing 50 mM imidazole, 250: Eluent containing 250 mM imidazole. (B) Purification of GOase M3 and its variants. M3: GOase M3, V1: variant GOase V1 (T406R), V2: variant GOase V2 (T406R/K330R), V3: variant GOase V3 (T406R/K330R/Y329F)

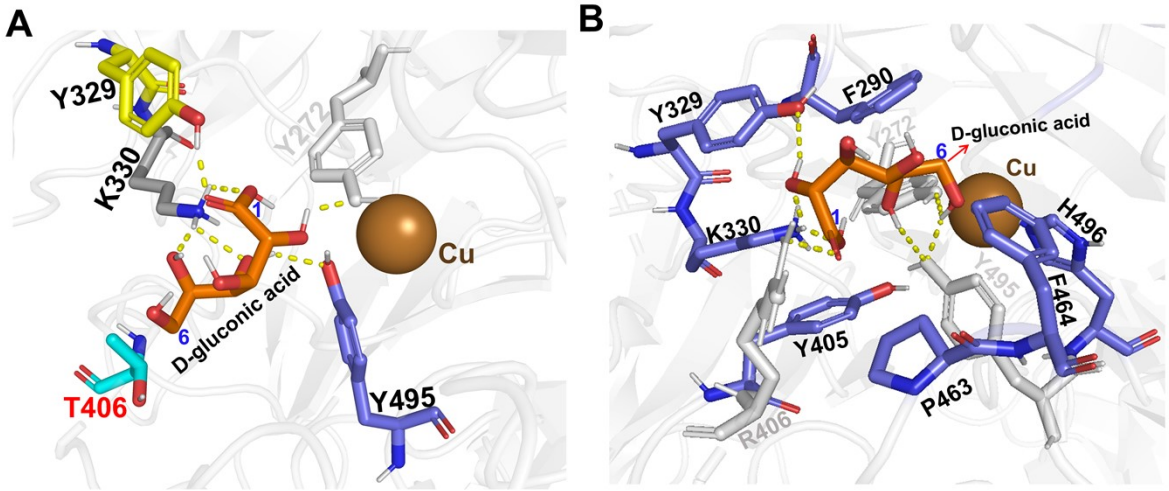

Figure S2. (A) Binding mode of open-chain D-gluconic acid in the active pocket of GOase M3 from molecular docking. The protein structure and catalytic residue component (Y272) are colored in white. The substrate D-gluconic acid is colored in orange. The hydrogen bonds are shown in yellow dashed lines. The mutation is labeled in red. The blue numbers '1' and '6' indicate the C-1 and C-6 positions of the substrate molecule, respectively. (B) Binding mode of open-chain D-gluconic acid in the active pocket of GOase V1 (T406R). The protein structure and binding residues (Y272, Y495, and R406) are colored in white. The substrate D-gluconic acid is colored in orange. Hydrogen bonds are shown as yellow dashed lines. The mutations are colored in blue. The blue numbers '1' and '6' indicate the C-1 and C-6 positions of the substrate molecule, respectively.

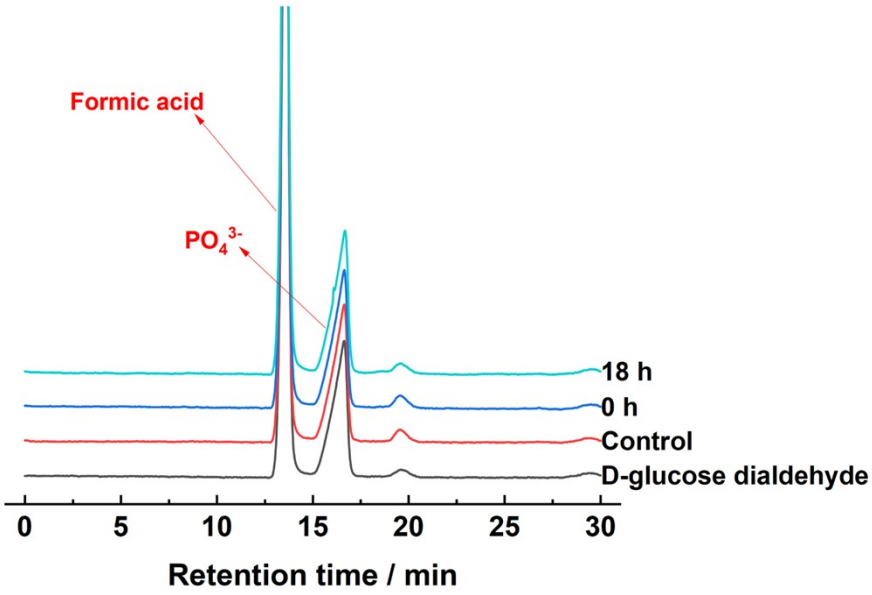

Figure S3. HPLC determination of production from D-glucose dialdehyde catalyzed by GOase V3. 0.5 mg mL^{-1} D-glucose dialdehyde, 5 mg mL^{-1} GOase V3, 1 mg mL^{-1} catalase, and 1 mg mL^{-1} HRP in 1.2 mL PBS buffer (100 mM, pH 7.5) at room temperature with continuous stirring (600 rpm) and oxygenation. The control included HRP and catalase, without GOase.

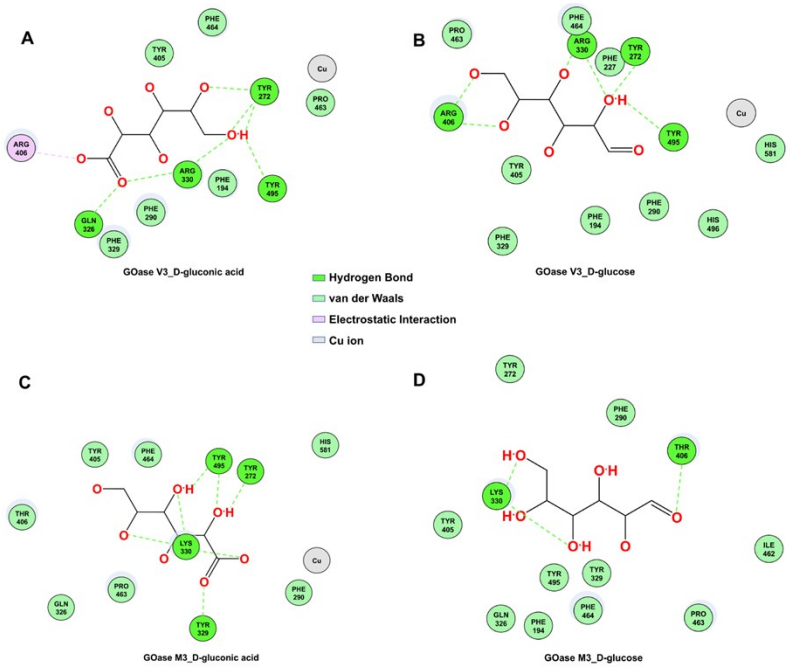

Figure S4. Interactions between GOase V3 and D-gluconic acid (A), GOase V3 and D-glucose (B), GOase M3 and D-gluconic acid (C) and GOase M3 and D-glucose (D).

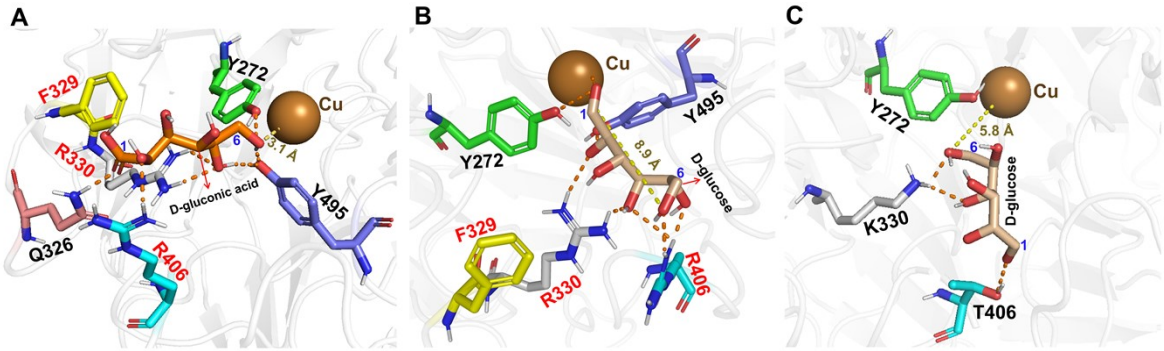

Figure S5. The interaction modes of (A) GOase V3 and open-chain D-gluconic acid, (B) GOase V3 and open-chain D-glucose, and (C) GOase M3 and open-chain D-glucose were obtained from molecular docking. D-gluconic acid is shown in orange, and D-glucose is shown in pink. Hydrogen bonds are shown in orange dashed lines. The bonds between Cu and the C-6 position of D-gluconic acid and D-glucose are shown in yellow dashed lines, around which the numbers indicate the distances (Å). The mutant residues are labeled in red. The blue numbers '1' and '6' indicate the C-1 and C-6 positions of the substrate molecule, respectively.

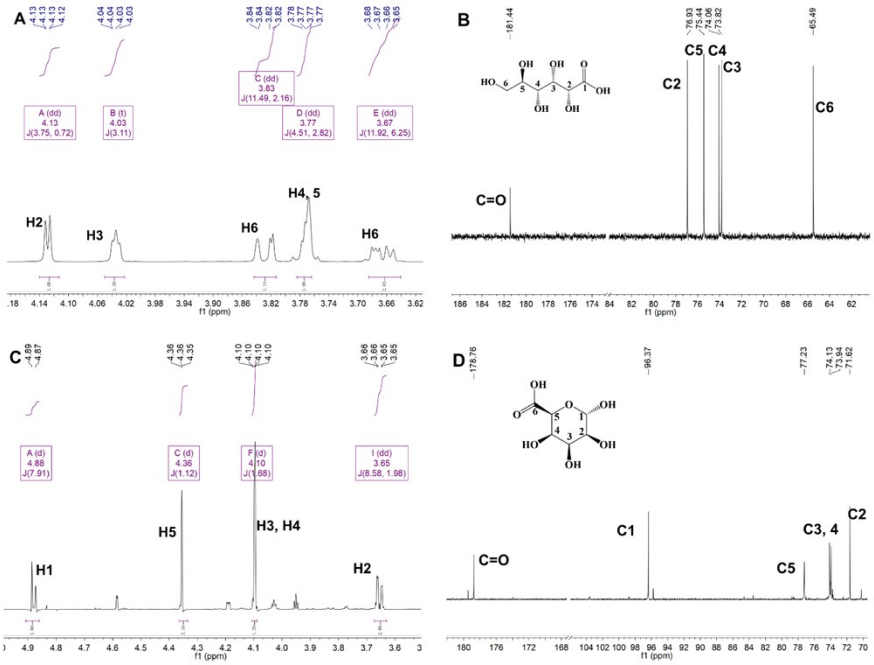

Figure S6. (A) NMR hydrogen spectrum of D-gluconic acid. (B) NMR carbon spectrum of D-gluconic acid. (C) NMR hydrogen spectrum of L-guluronic acid. (D) NMR carbon spectrum of L-guluronic acid. D-gluconic acid: ^{1}H NMR (600 MHz, D_2O) δ 4.13 (d, 3.75, 1H), 4.03, (t, 3.11, 1H), 3.83 (dd, 11.49, 2.16, 1H), 3.77 (m, 2H), 3.67 (dd, 11.92, 6.25, 1H), ^{13}C NMR (151 MHz, D_2O) δ 181.44 (COOH), 76.93 (CH), 75.44 (CH), 74.06 (CH), 73.82 (CH), 65.49 (CH); L-guluronic acid: ^{1}H NMR (600 MHz, D_2O) δ 4.88 (d, 7.91, 1H), 4.36 (d, 1.12, 1H), 4.10 (m 2H), 3.65 (dd, 8.58, 1.98, 1H), ^{13}C NMR (151 MHz, D_2O) δ 178.76 (COOH), 96.37 (CH), 77.23 (CH), 74.13 (CH), 73.94 (CH), 71.62 (CH).

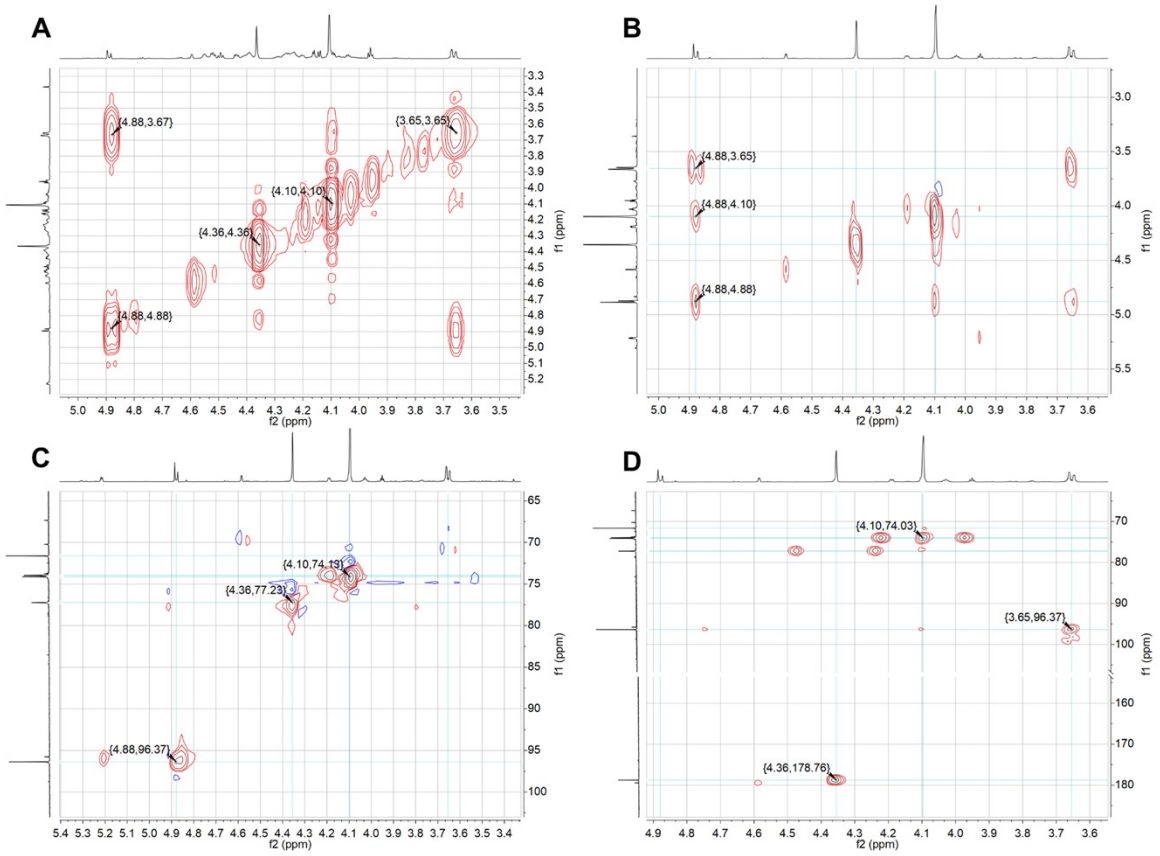

Figure S7. (A) ^{1}H-^{1}H COSY spectrum of L-guluronic acid. (B) ^{1}H-^{1}H TOCSY spectrum of L-guluronic acid. (C) ^{1}H-^{13}C HSQC spectrum of L-guluronic acid. (D) ^{1}H-^{13}C HMBC spectrum of L-guluronic acid. ^{1}H-^{1}H COSY: H-1 (δ_H 4.88, d, J = 7.91 Hz) to H-2 (δ_H 3.65, dd, J = 8.58, 1.98 Hz); ^{1}H-^{1}H TOCSY: H-1 (δ_H 4.88, d, J = 7.91 Hz) to H-2 (δ_H 3.65, dd, J = 8.58, 1.98 Hz); ^{1}H-^{13}C HSQC: H-1 (δ_H 4.88, d, J = 7.91 Hz) to C-1 (CH, δ_C 96.37), H-2 (δ_H 3.65, dd, J = 8.58, 1.98 Hz) to C-2 (CH, δ_C 71.62), H-3 (δ_H 4.10, m) to C-3 (CH, δ_C 74.13), H-4 (δ_H 4.10, m) to C-4 (CH, δ_C 73.94), H-5 (δ_H 4.36, d, J = 1.1 Hz) to C-5 (CH, δ_C 77.23); ^{1}H-^{13}C HMBC: H-2 (δ_H 3.65, dd, J = 8.58, 1.98 Hz) to C-1 (CH, δ_C 96.37), H-5 (δ_H 4.36, d, J = 1.1 Hz) to C-6 (COOH, δ_C 178.76).

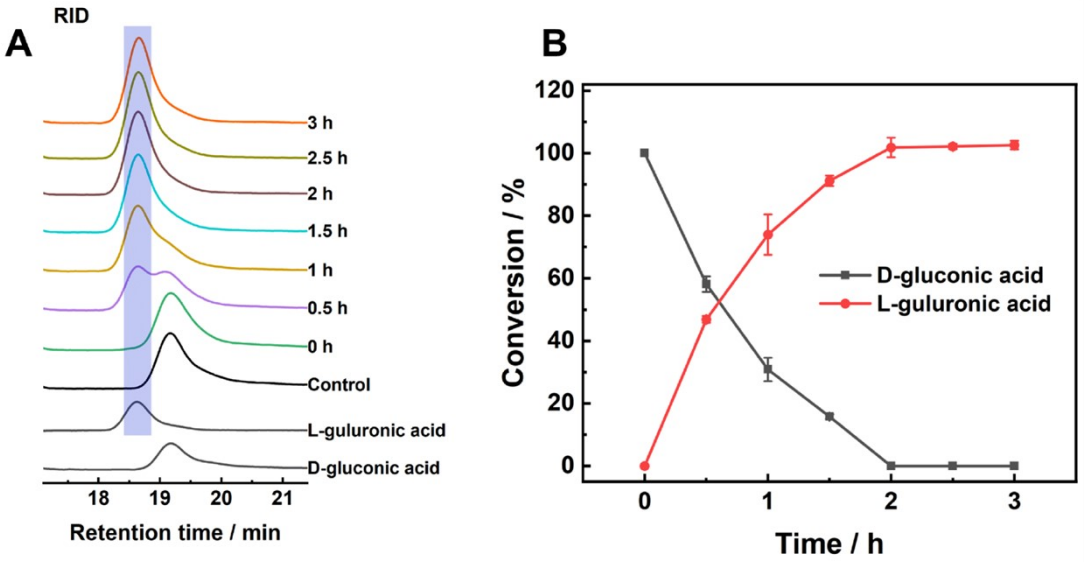

Figure S8. (A) HPLC determination and (B) Time course of L-guluronic acid production from D-gluconic acid catalyzed by GOase V3. 40 mM D-gluconic acid, 5 mg mL^{-1} GOase V3, 1 mg mL^{-1} catalase, and 1 mg mL^{-1} HRP in 1.2 mL PBS buffer (100 mM, pH 7.5) at room temperature with continuous stirring (600 rpm) and oxygenation. The control included HRP and catalase, without GOase. Values shown represent the mean ± s.d. of triplicate determinations.

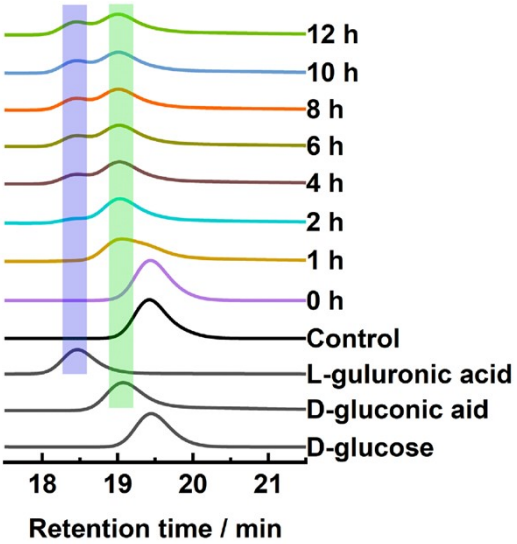

Figure S9. HPLC determination L-guluronic acid production from D-glucose catalyzed by GOx and GOase V3. 40 mM D-glucose, 0.5 mg mL^{-1} GOx, 5 mg mL^{-1} GOase V3, and 1 mg mL^{-1} catalase in 1.2 mL PBS buffer (100 mM, pH 7.0) at room temperature with continuous stirring (600 rpm) and oxygenation. The control included catalase, without GOase and GOx.

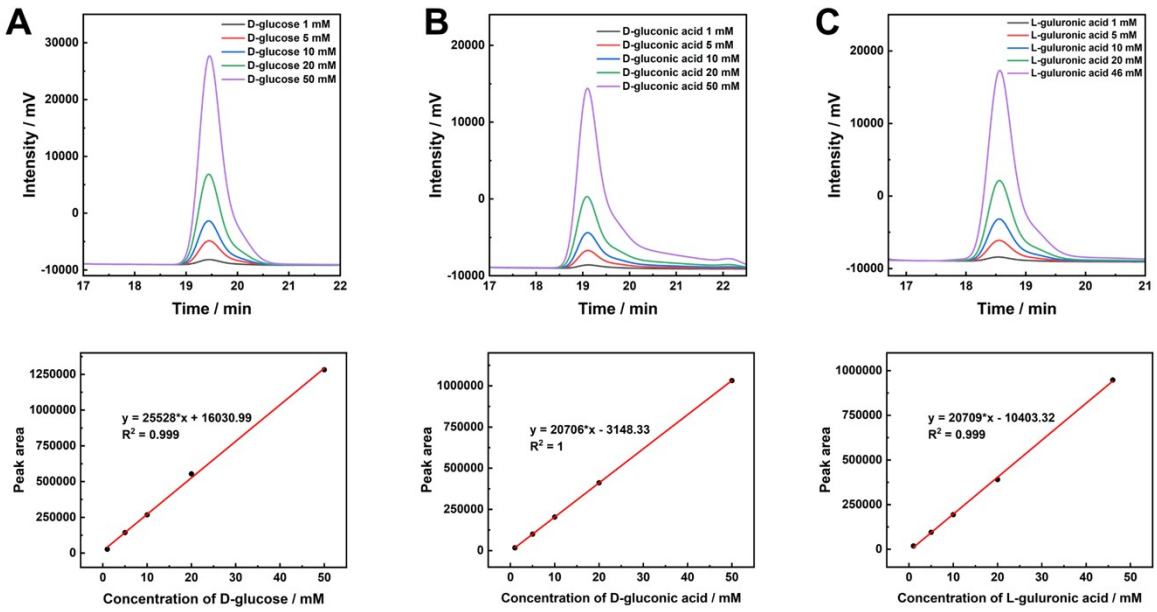

Figure S10. HPLC determinations and standard curves of D-glucose (A), D-gluconic acid (B) and L-guluronic acid (C).

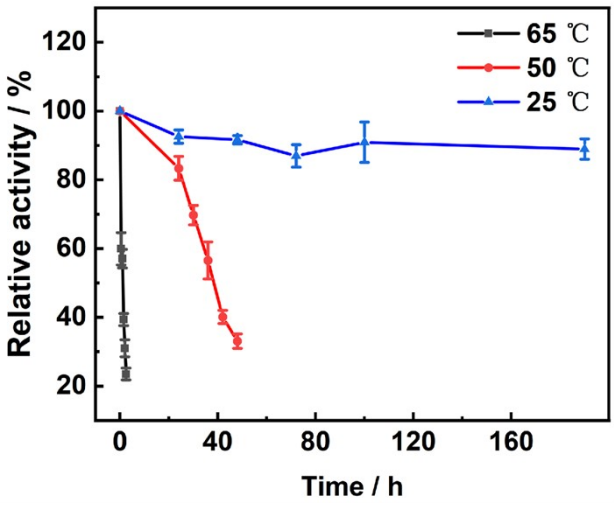

Figure S11. Half-life determination of GOase V3.

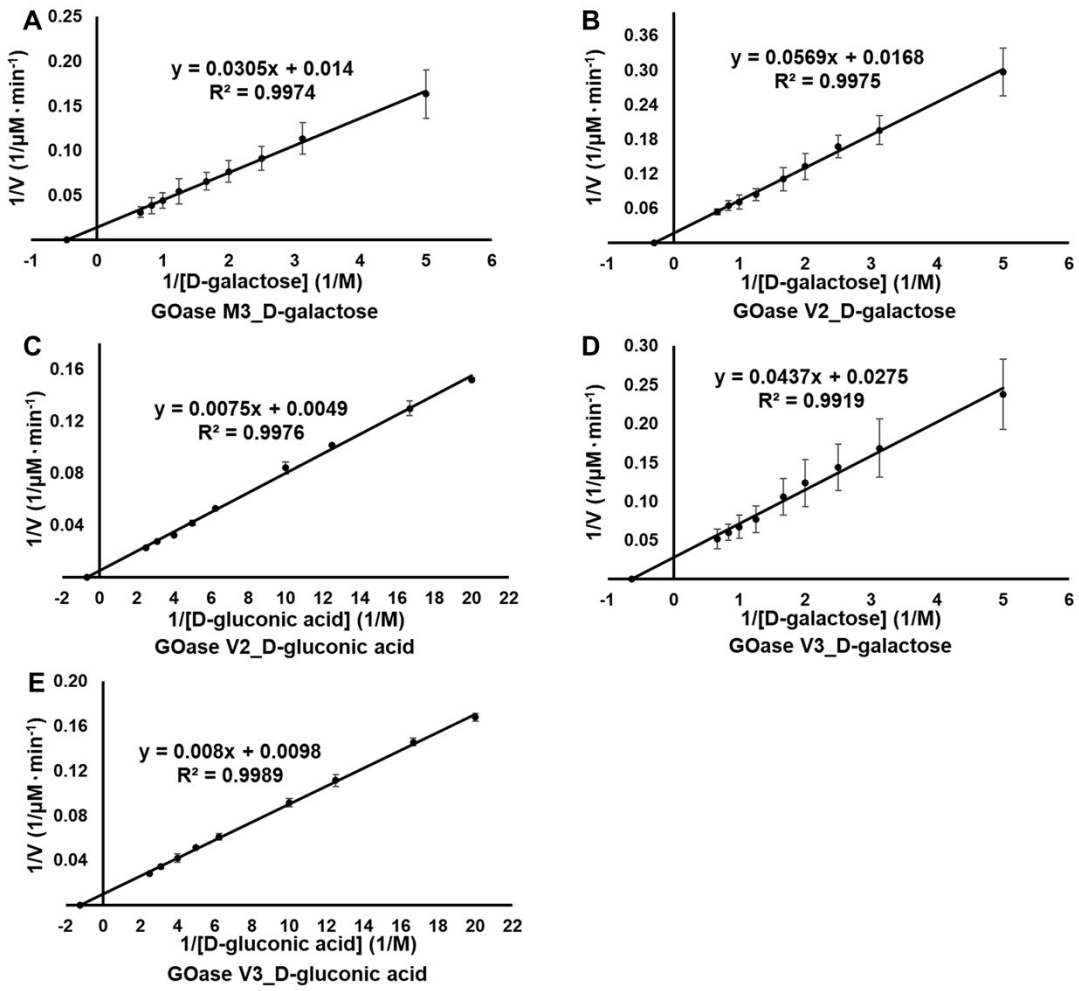

Figure S12. (A) The Lineweaver-Burk plot of GOase M3 catalyzing D-galactose. The Lineweaver-Burk plots of GOase V2 catalyzing D-galactose (B) and D-gluconic acid (C). The Lineweaver-Burk plots of GOase V3 catalyzing D-galactose (D) and D-gluconic acid (E).