Supplementary Material

Targeted Directed Evolution of an α-L-Rhamnosidase on Hesperidin through Six-condon Combinatorial Mutagenesis

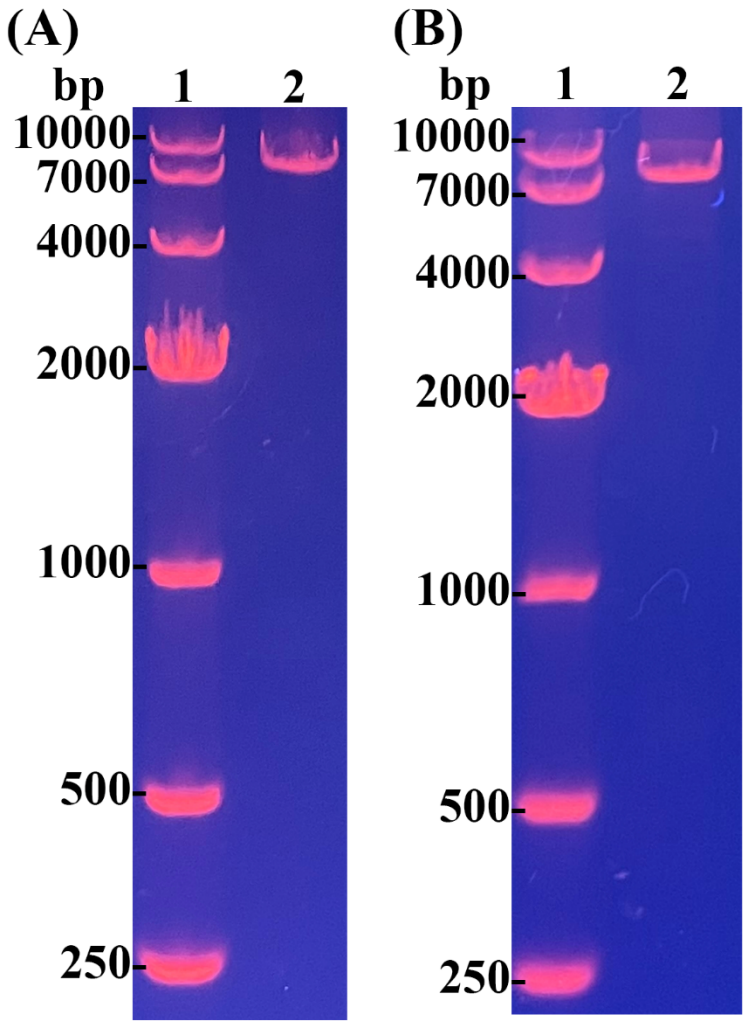

Figure S1. Agarose gel for whole-plasmid PCR products for constructing the mutant library of six-codons combinatorial mutagenesis. In the figures **(A)** and **(B)**, lane 1 represents the molecular weight standard of DNA, Lane 2 in **(A)** represents TM1 whole plasmid PCR products; Lanes 2 in **(B)** is TM2 whole plasmid PCR products.

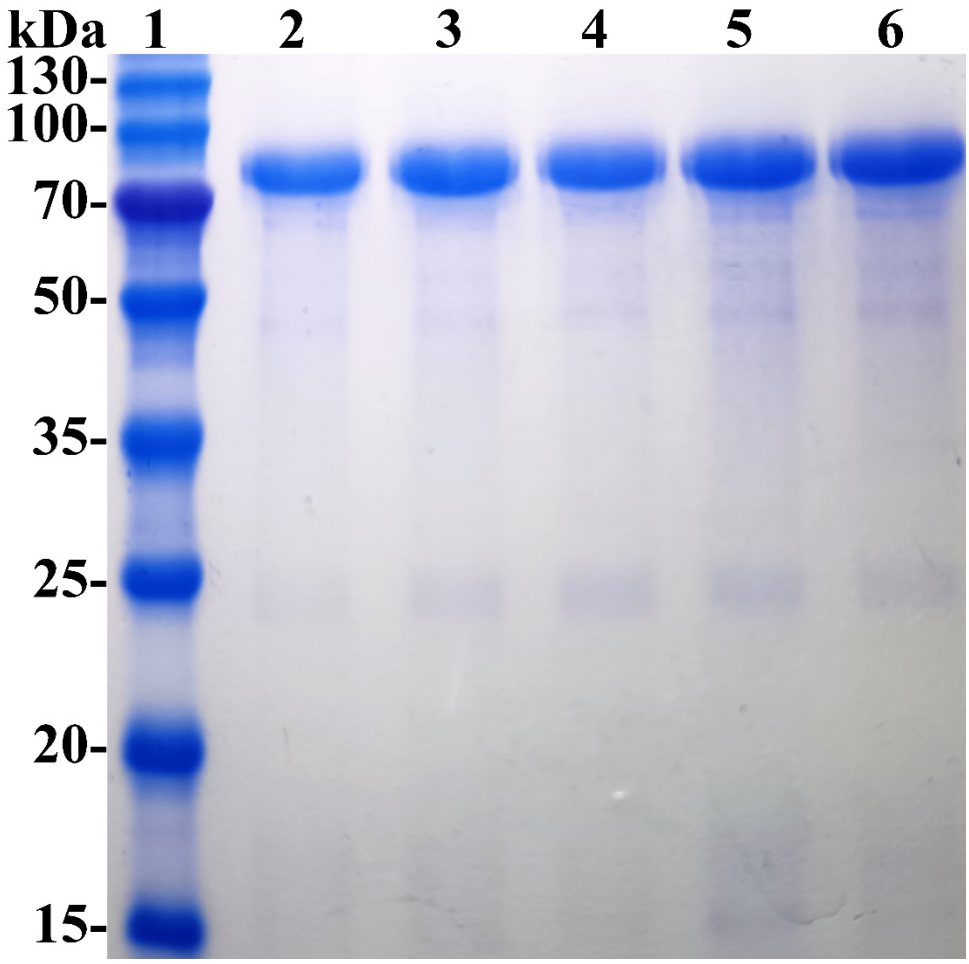

Figure S2. SDS-PAGE (15%) for purification of BtRha78A wild type and better mutants on hesperidin. Lines 1-6 indicate protein molecular weight standard marker, purified BtRha78A-WT, TM1-6-F5, TM1-6-H6, TM1-7-G, and TM1-8-F9, respectively.

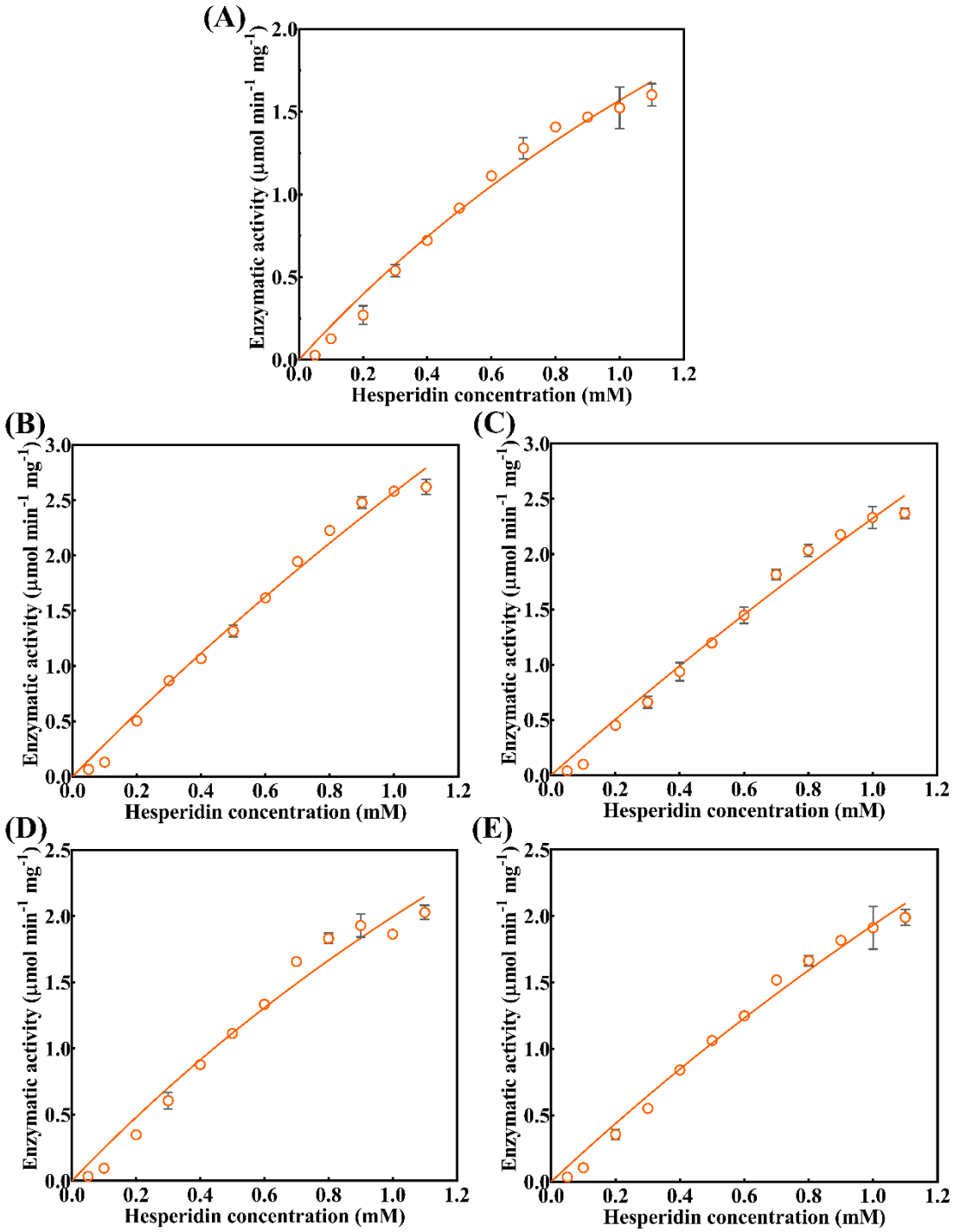

Figure S3. Enzyme kinetic curves of BtRha78A wild type and better mutants on hesperidin. (A). Enzyme kinetic curve of BtRha78A wild type on hesperidin. **(B).** Enzyme kinetic curve of BtRha78A TM1-6-F5 on hesperidin. **(C).** Enzyme kinetic curve of BtRha78A TM1-6-H6 on hesperidin. **(D).** Enzyme kinetic curve of BtRha78A TM1-7-G1 on hesperidin. **(E).** Enzyme kinetic curve of BtRha78A TM1-8-F9 on hesperidin. All reactions were performed in triplicate, and error bars represent the standard deviations of mean.

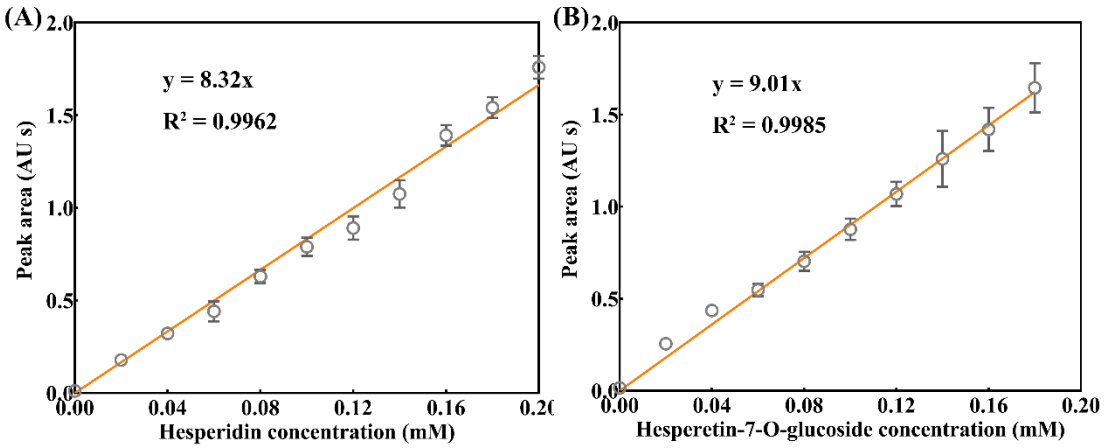

Figure S4. Standard curves for hesperidin and hesperetin-7-O-glucoside by HPLC. (A). Standard curve for hesperidin; **(B)**. Standard curve for hesperetin-7-O-glucoside. All reactions were performed in triplicate, and error bars represent the standard deviations of mean.