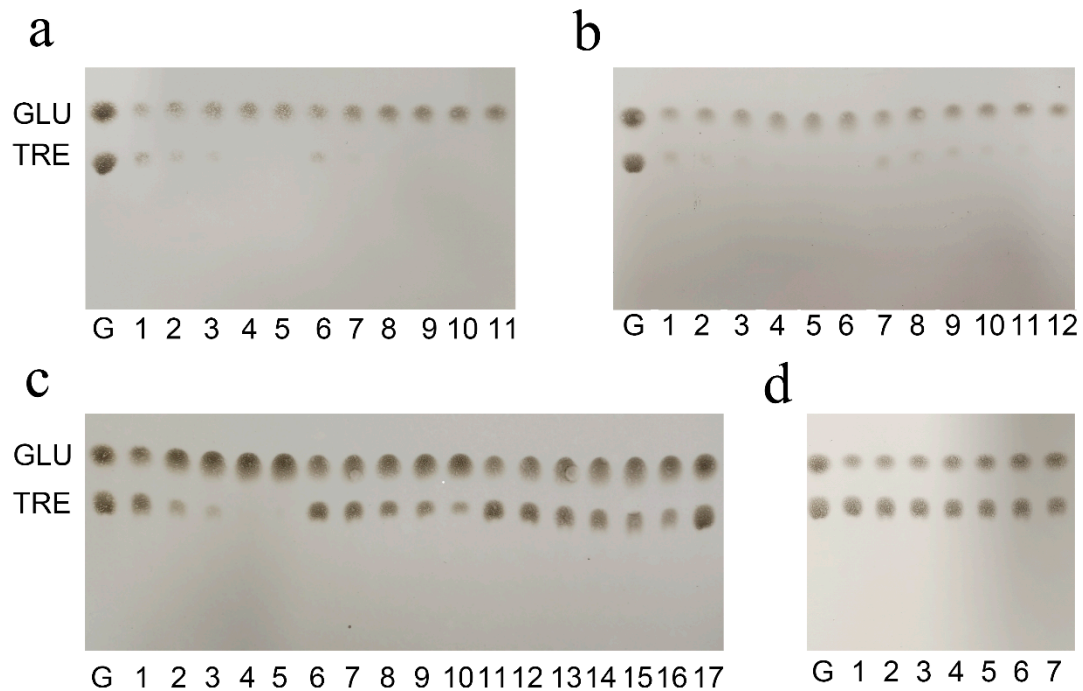


**Supplementary Figure S1** SDS-PAGE analysis of the MpTre15A mutants

Lane M, molecular size marker (Unstained Protein Molecular Weight Marker: 116, 66.2, 45, 35, 25 kDa); lane 1, crude enzyme of E392Q mutant; lane 2, purified enzyme of E392Q mutant after Ni-IDA column; lane 3, crude enzyme of E557Q mutant; lane 4, purified enzyme of E557Q mutant.



**Supplementary Figure S2** Thin layer chromatography (TLC) analysis of the hydrolytic products of trehalose. Panel a and b, TLC analysis of trehalose hydrolysates at different temperatures with 5 mg/mL trehalose. Panel a: Lane G indicates glucose and trehalose mixed standard; Lane 1 to Lane 5 indicate the hydrolytic product of trehalose at 50 °C for 1, 3, 5, 10, and 60 min, respectively; Lane 6 to Lane 11 indicate the hydrolytic product of trehalose at 37 °C for 5, 15, 30, 45, 60, and 120 min, respectively. Panel b: Lane G indicates glucose and trehalose mixed standard; Lane 1 to Lane 6 indicate the hydrolytic product of trehalose at 25 °C for 10, 30, 45, 60, 90, and 120 min, respectively; Lane 7 to Lane 12 indicates the hydrolytic product of trehalose at 4 °C for 20, 40, 60, 120, 180, and 240 min, respectively. Panel c and d, TLC analysis of trehalose hydrolysates at different temperatures with 50 mg/mL trehalose. Panel c: Lane G indicate glucose and trehalose mixed standard; Lane 1 to Lane 5 indicate the hydrolytic product of trehalose at 50 °C for 15, 30, 45, 60, and 120 min, respectively; Lane 6 to Lane 10 indicate the hydrolytic product of trehalose at 37 °C for 15, 30, 45, 60, and 120 min, respectively; Lane 11 to Lane 16 indicate the hydrolytic product of trehalose at 25 °C for 30, 60, 90, 120, 180, and 240 min, respectively; Lane 17 indicates glucose and trehalose mixed standard. Panel d: Lane G indicates glucose and trehalose mixed standard; Lane 1 to Lane 7 indicate the hydrolytic product of trehalose at 4 °C for 30, 60, 90, 120, 180, 240, and 360 min, respectively.