

Supporting Information

Table S1: Active Site Titration Data for MUTYH and Variants

Enzyme	Total protein (mg/L)	Total active yield (µg/L)
WT MUTYH	1.0, 1.1, 1.1, 0.9, 1.0, 2.0, 0.9, 0.9, 1.3	9, 10, 9, 7, 9, 20, 10, 15
R295C MUTYH	0.9, 0.5, 0.2, 0.5, 0.4, 0.4, 0.3, 0.9	0.2, 0.4, 0.6, 0.7, 0.8, 0.8, 0.5, 0.6
P281L MUTYH	0.6, 0.6, 0.3, 0.4, 0.6	<0.01
Q324H MUTYH	1.2, 1.1, 0.9, 1.1, 1.1, 1.0, 2.0, 1.1, 0.7, 0.8, 1.2, 1.3	6.7, 16, 7, 12, 8, 7.7, 14, 12, 8, 8, 7.7, 10
P502L MUTYH	0.8, 0.3, 0.9, 0.9, 1.3, 0.5	10, 6, 20, 14, 25,
R520Q MUTYH	0.3, 0.3, 0.6, 0.4, 0.5, 0.2, 0.4, 0.2, 0.6, 0.6	0.7, 0.4, 0.5, 2.0, 0.4, 0.2, 0.5, 0.2, 1.2, 0.4, 1.3, 0.9, 1.0

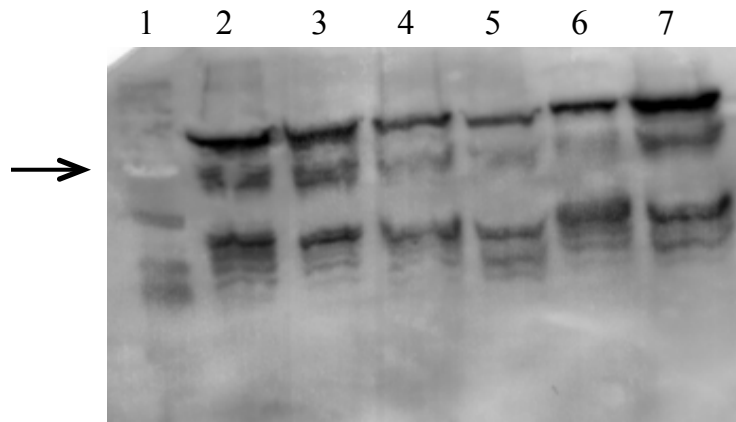


Figure S1: Western blot analysis of the overexpression of WT MUTYH and MUTYH missense variants located in protein functional domains. From left: lane 1: marker, with highlight at 80 kDa (indicated by arrow); lane 2: WT MUTYH (~102 kDa); lane 3: Q324H MUTYH; lane 4: P281L MUTYH; lane 5: R520Q MUTYH; lane 6: R295C MUTYH; and lane 7: P502L MUTYH. Similar amounts of cell lysate were added to each well.

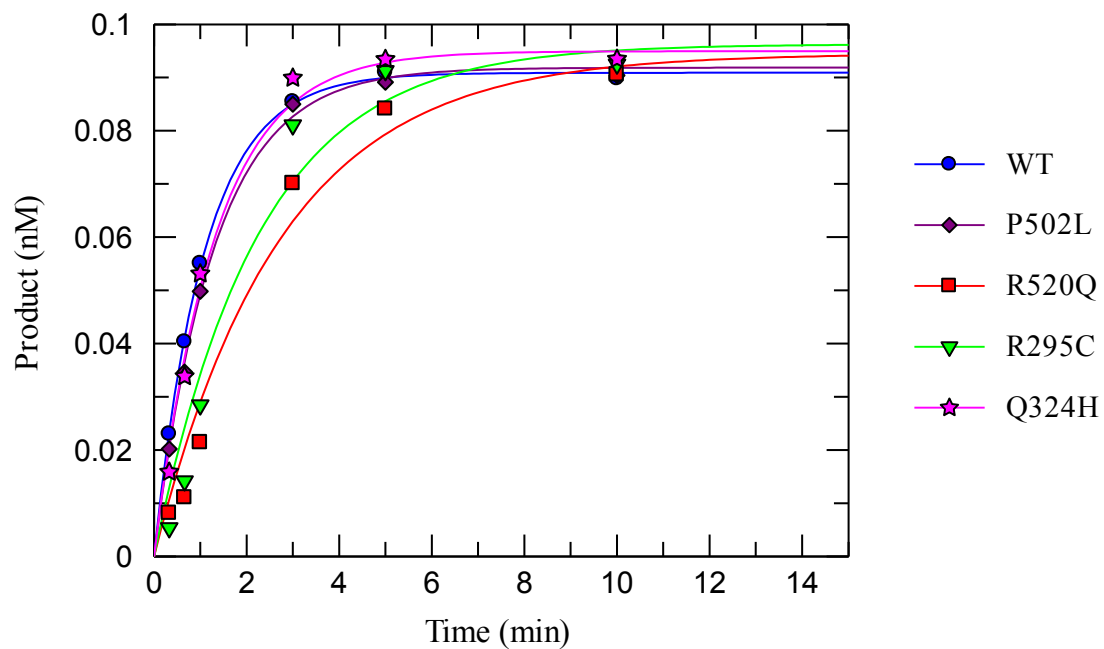


Figure S2. Comparison of Adenine Glycosylase Activity of WT MUTYH relative to MUTYH Variants. Shown are representative plots from adenine glycosylase assays performed under single-turnover conditions of WT MUTYH and MUTYH missense variants with an OG:A-containing DNA substrate in buffer containing 60 mM NaCl at 37 °C. The values for the rate constant k_2 were determined from at least three separate experiments for each enzyme (from different enzyme preparations) and were averaged (Table 1).