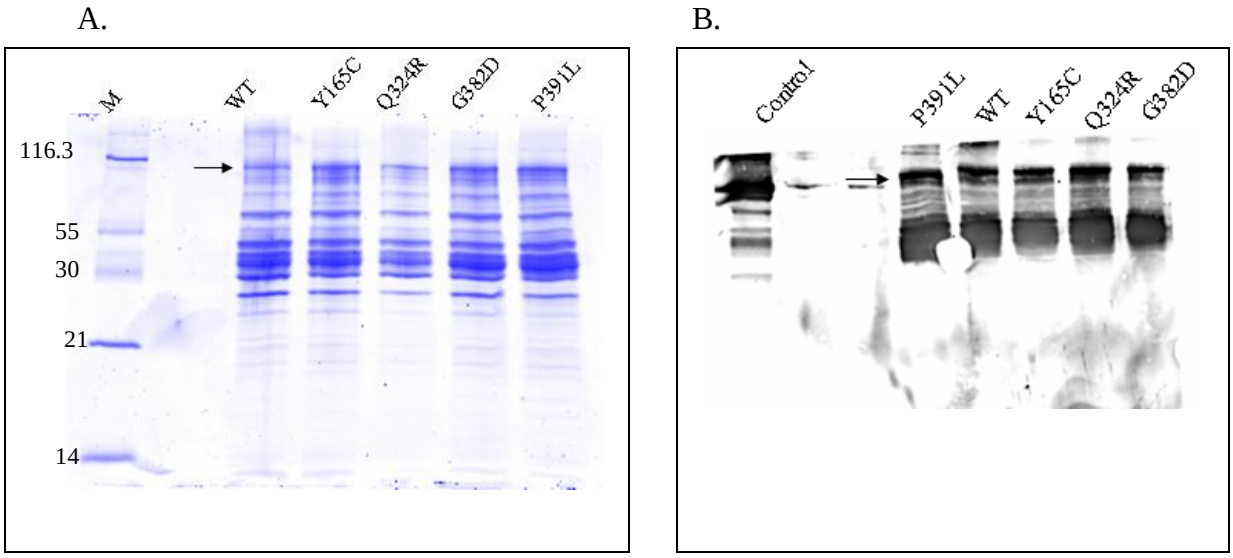
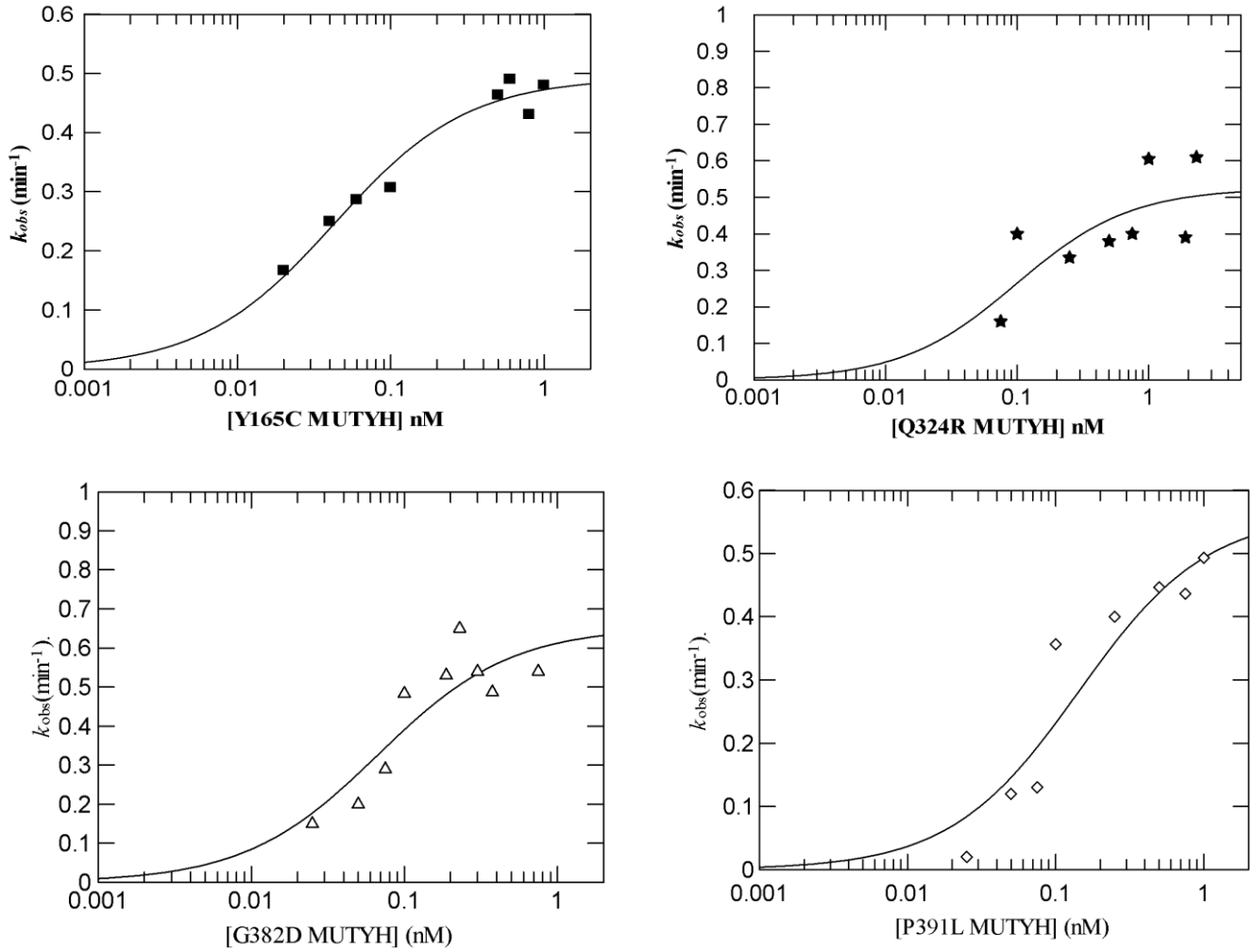


Supplementary Figure 1: Analysis of purification of N-terminal MBP tagged recombinant WT MUTYH and variants. The same samples were run on two separate 12% SDS polyacrylamide gels. **(A)** Sypro orange staining reveals all protein bands. The arrow indicate full-length MBP-MUTYH. M = Marker P-6649 (Invitrogen) **(B)** Western blot analysis of the same samples using primary monoclonal antibody against the MBP tag. Partially purified *E.coli* MBP-MutY was used as a control. A total of one ug (measured by Bradford assay) of total protein was loaded into each well for WT MUTYH and the variants. The percent purity of MUTYH and variants was determined to be approximately 30% using ImageQuant software. The MW of the major bands correspond to free MBP, indicating premature termination of translation after the MBP tag.



Supplementary Figure 2: Representative binding data of MUTYH variants. Rate of adenine removal (k_{obs}) was determined at 37°C at 150 mM buffer NaCl concentration. Reaction conditions included an OG:A mismatch- containing duplex DNA substrate (0.01 nM) and enzyme concentrations between 0.02 and 2 nM. The lines represent the fit to a single binding isotherm to determine the K_d values: Y165C, 0.04 nM; Q324R, 0.1 nM; G382D, 0.07 nM; P391L, 0.14 nM. These values are similar to the K_d determined for WT MUTYH of 0.3 ± 0.1 nM.