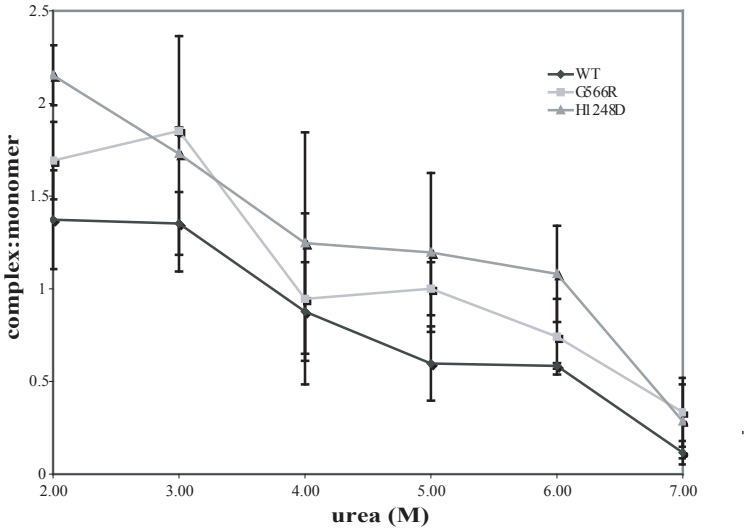
Fig. S1. Urea denaturation of wild-type, G566R, and H1248D mutants. Stability of the hMSH2-hMSH6 complex was analyzed at increasing concentrations of urea (2-7 M) and examined on a native gel. The ratio of protein in a complex (heterodimer or higher order multimer) versus a monomeric form is plotted for each urea concentration. The urea-dependent denaturation of wild-type, G566R, and H1248D mutants appear similar. Error bars show +/- one S.D. Each experiment was performed in triplicate.

Fig. S2. Steady-state ATPase activity of hMSH6 missense mutants. ATPase velocity determined for multiple concentrations of protein incubated for 30 min at 37°C with or without 240nM G/T heteroduplex DNA, and increasing amounts of ATP, of which 17nM was radiolabeled. The amount of released γ-phosphate was determined by charcoal binding. Kinetic constants were determined by fitting the data to the Michaelis-Menten equation (Table 1). Error bars show +/- one S.D. Each experiment was performed a minimum of three times.

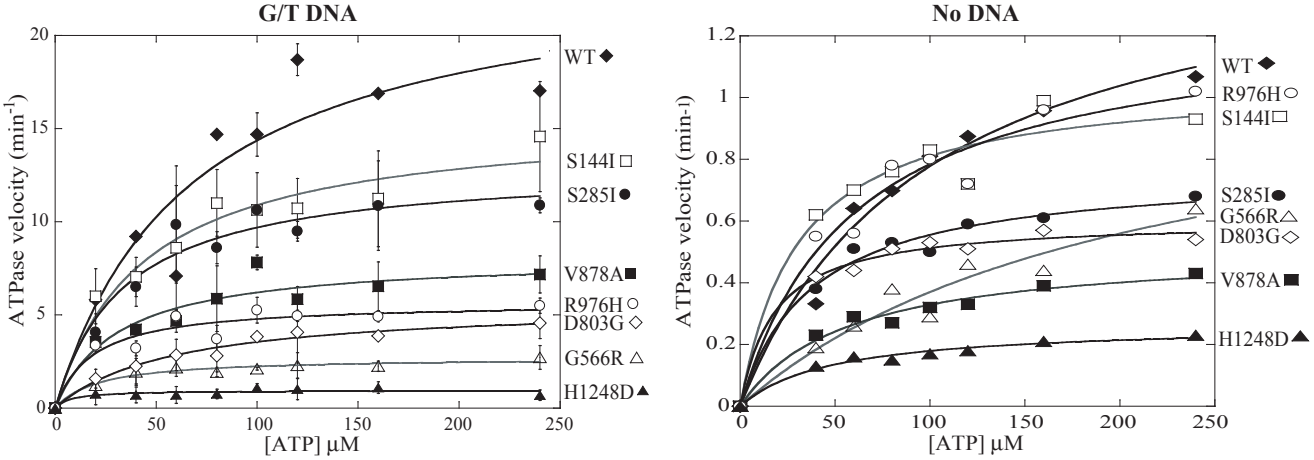
Fig. S3. Mismatch binding of WT and hMSH6 missense mutants. An overlay of Biacore surface plasmon resonance sensorgrams displaying the association curves of 50nM WT and hMSH6 missense mutant-containing heterodimers with a 41-bp heteroduplex oligonucleotide. Multiple concentrations of each protein were used to determine kinetic constants (see Table 2).

Fig. S4. Partial trypsin proteolysis of hMSH2-hMSH6 missense mutants. *A*, Wild-type hMSH2-hMSH6 was partially digested with increasing amounts of trypsin and analyzed by western blotting with polyclonal anti-hMSH6 and anti-hMSH2 antibodies to determine the identity of proteolytic products. *B*, Silver-stained acrylamide gels showing the partial trypsin proteolysis products of S144I, V878A, and H1248D mutants.

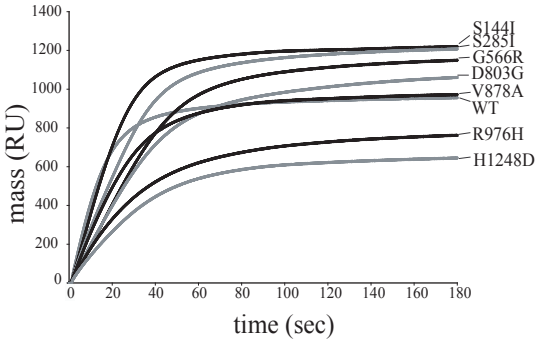
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4