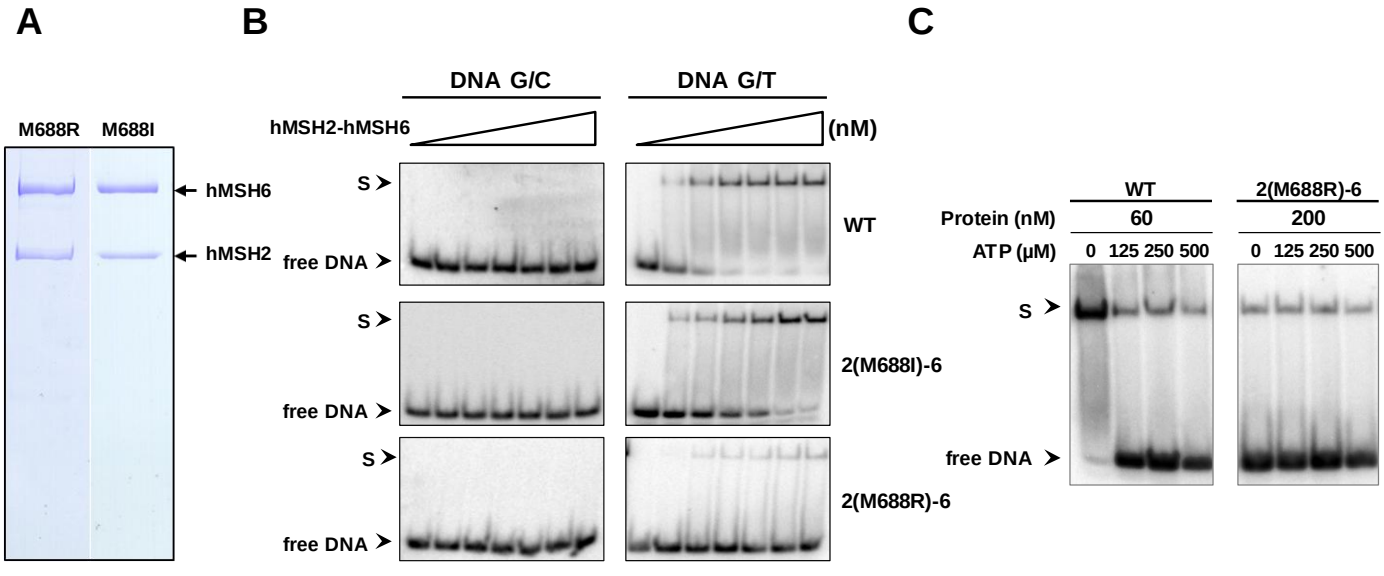
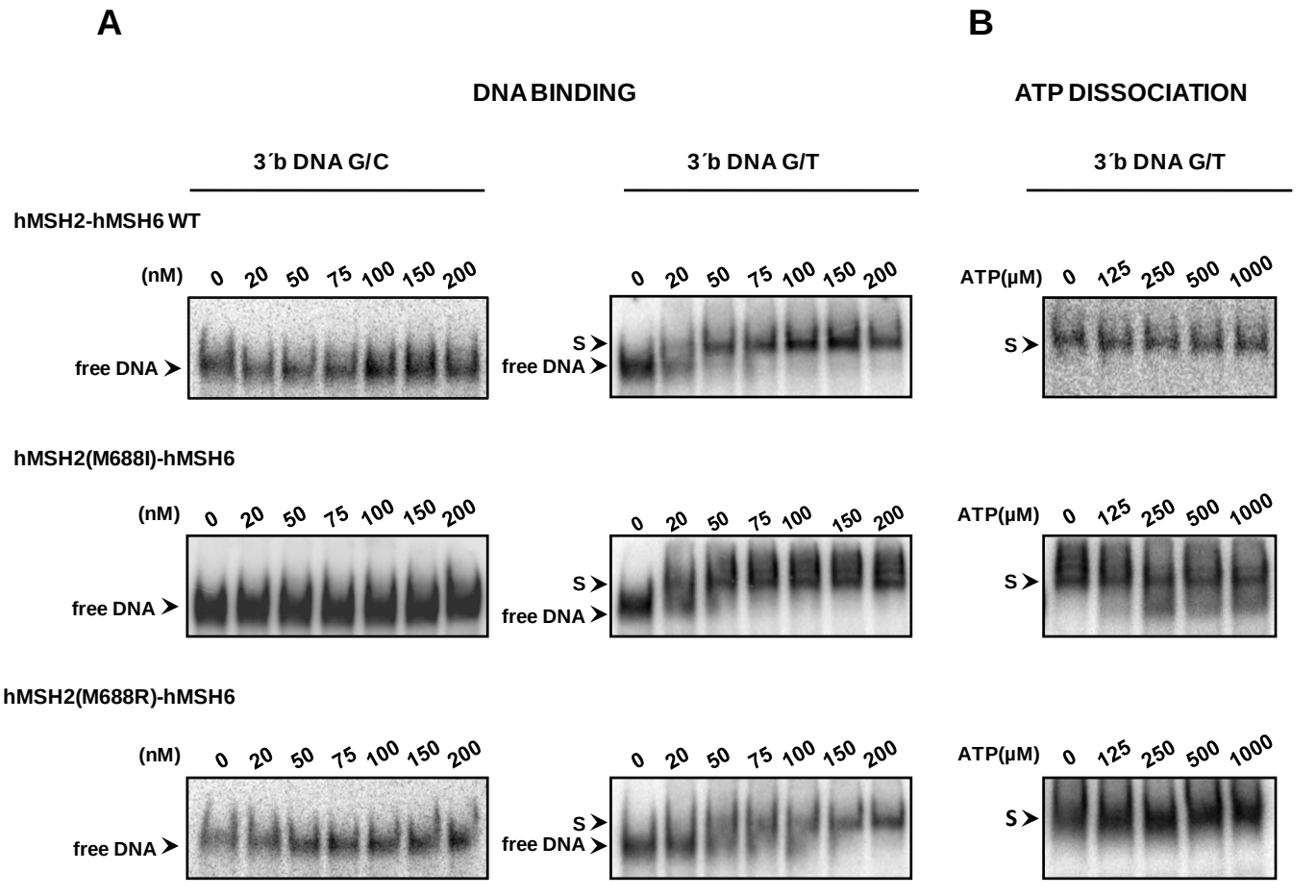
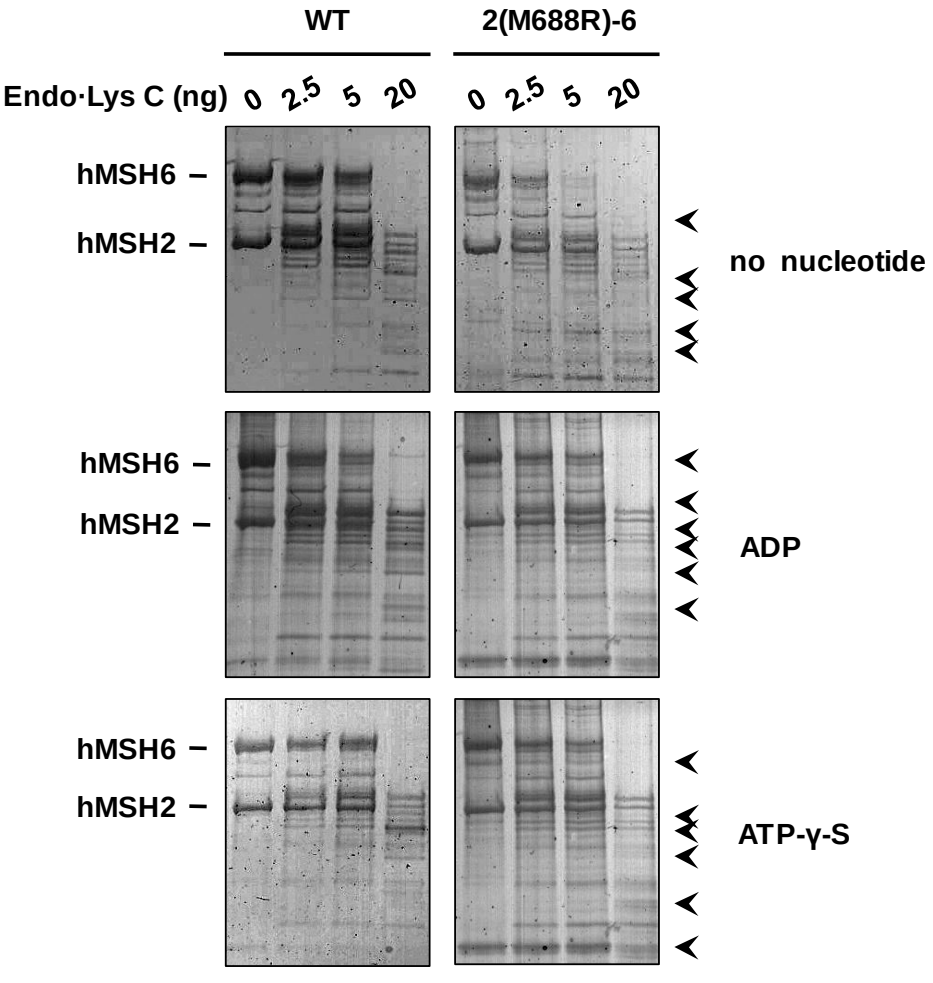


Supplementary Figure 1. DNA binding analysis and ATP-induced dissociation of hMSH2-hMSH6 from mismatched DNA by electrophoretic mobility shift assay.

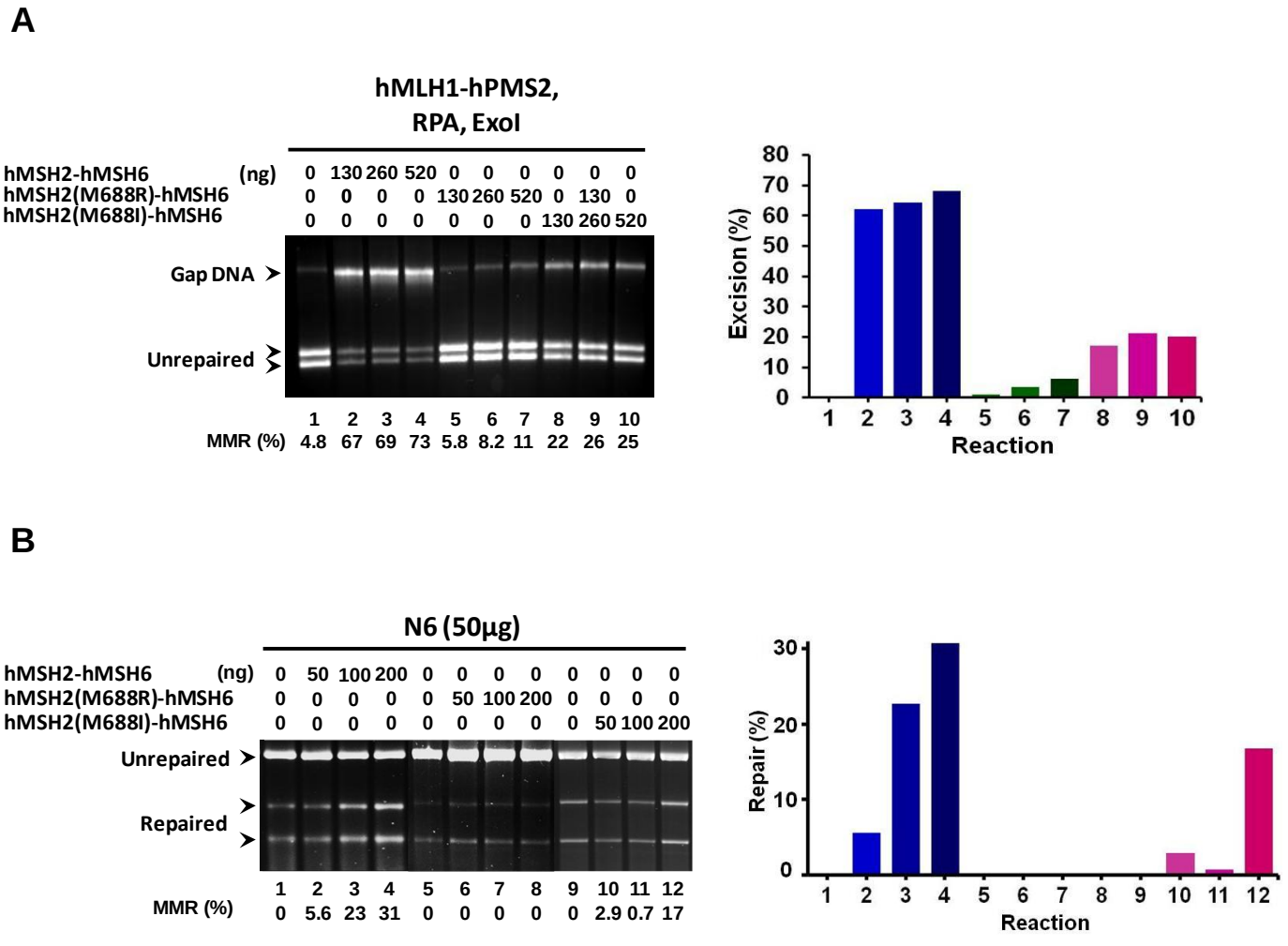
(**A**) Representative coomassie stained gel of purified hMSH2(M688R)-hMSH6 and hMSH2(M688I)-hMSH6. (**B**) The ability to recognize and bind to homo(G/C)- and hetero(G/T)- duplex DNA was tested. 10 fmol of DNA was incubated with increasing concentrations of hMSH2-hMSH6 (0-200nM). Left column: Binding to duplex DNA. Right column: G/T mismatched DNA binding. S indicates specific gel shift protein-DNA complex. (**C**) hMSH2-hMSH6 WT (60nM) dissociation was used as positive control. hMSH2(M688R)-hMSH6 was tested using different concentrations: 60nM, 100nM, 150nM and 200nM, because of the reduced mismatch DNA binding previously observed (only 200nM is shown). Increasing ATP concentrations were added after the formation of the DNA·hMSH2-hMSH6 complex. **S**: DNA·hMSH2-hMSH6 complex



Supplementary Figure 2. DNA binding and ATP induced-DNA dissociation of hMSH2-hMSH6 in the presence of duple DNA (b-G/C) or mismatched (b-G/T) DNA with both 3´ ends blocked with biotin-streptavidin. (**A**) hMSH2-hMSH6 binding to biotin-streptavidin DNA blocked-ends. All binding reactions were performed in the presence of ADP (25μM), $MgCl_2$ (2mM), streptavidin (850nM) and increasing concentrations of protein complexes (0-200nM). Left column: hMSH2-hMSH6 binding to duplex DNA. Right column: Binding to a G/T mismatched DNA. (**B**) ATP-induced release of hMSH2-hMSH6 from mismatched DNA containing biotin-streptavidin blocked ends. The reactions were performed using 200nM hMSH2-hMSH6. Increasing ATP concentrations were added after the DNA binding. **S**: hMSH2-hMSH6·DNA complex.



Supplementary Figure 3. Limited endoproteinase-Lys-C proteolysis of purified hMSH2-hMSH6 in the presence/absence of adenosine nucleotides. Partial proteolysis of hMSH2-hMSH6 unbound to adenosine nucleotide, prebound to ADP or ATP-γ-S using 2.5ng, 5ng, 20ng or 80ng (not shown) of endo Lys-C. Conformational changes expose and/or obscure protease sites on the surface of a protein complex. hMSH2-hMSH6 proteolytic products were analyzed by SDS-PAGE and silver staining. Differing peptide products as a result of different conformations are marked with arrowheads.



Supplementary Figure 4. Mismatch-dependent excision and complete mismatch repair analysis of hMSH2-hMSH6, hMSH2(M688R)-hMSH6, hMSH2(M688I)-hMSH6. (A) Representative mismatch-dependent excision analysis. The formation of a single stranded DNA (ssDNA) excision tract was examined in the presence of purified hMLH1-hPMS2 (400 fmol), RPA (800 fmol) and Exo I (5 fmol), and *wild type* or mutant hMSH2-hMSH6 as shown above gel in a 20 ml reaction (see Methods). The formation of ssDNA was scored as previously described (see ref. 34,35). Quantitation of the gel results (left panel) is shown in the right panel. **(B)** Representative complete mismatch repair analysis. The formation of a complete MMR repair product was examined in the presence of purified hMLH1-hPMS2 (400 fmol), RPA (800 fmol) and Exo I (5 fmol), and *wild type* or mutant hMSH2-hMSH6 as shown above gel in a 20 ml reaction (see Methods). The formation of a complete MMR repair product was scored as previously described (see ref. 34,35). Quantitation of the gel results (left panel) is shown in the right panel.