TABLE S1.
95% confidence intervals for $S_{0.5}$ adenosine nucleotide cross-linking affinity

Protein		[α-P^{32}]-ADP		[α-P^{32}]-ATP		[γ-P^{32}]-ATP		[γ-S^{35}]-ATP	
		- Mg	+ Mg	- Mg	+ Mg	- Mg	+ Mg	- Mg	+ Mg
WT	hMSH6	8-10	ND	0.02-0.05	> 1	0.03-0.05	ND	0.27-0.31	5-8
	hMSH2	6-7	2.0-2.2	0.3-1.4	0.08-0.21	0.5-1.2	ND	5-8	> 15
2KA/6	hMSH6	ND	ND	0.010-0.028	> 1	0.04-0.05	ND	0.11-0.13	> 9
	hMSH2	ND	ND	ND	ND	ND	ND	ND	ND
2/6KA	hMSH6	ND	ND	ND	0.2-3.1	ND	0.07-0.16	ND	0.4-0.7
	hMSH2	0.4-1.4	0.3-1.0	0.1-0.5	0.01-0.07	0.02-0.05	> 4	0.04-0.07	0.1-0.3
2KA/6KA	hMSH6	ND	ND	ND	0.09-0.16	ND	0.03-0.12	ND	ND
	hMSH2	ND	ND	ND	ND	ND	ND	ND	ND

TABLE S2.
DNA mismatch binding and sliding clamp formation

Protein	k_{on} *(x10^{-3} nM^{-1}·sec^{-1})*	k_{off} *(x10^{-3} sec^{-1})*	$k_{off\cdot ATP}$ *(x10^{-1} sec^{-1})*	$k_{off\cdot ATP}$ (No Mg^{2+})[1] *(x10^{-1} sec^{-1})*	$k_{off\cdot ATP}$ (No Mg^{2+})[2] *(x10^{-1} sec^{-1})*
WT	9.3	5.6	7.5	2.32	0.22
2KA/6	13.8	8.6	0.4	0.11	0.04
2/6KA	8.6	6.9	0.6	0.14	0.03
2KA/6KA	14.1	6.3	0.04	0.10	0.04

[1] +Mg^{2+} in binding buffer, - Mg^{2+} in ATP wash buffer
[2] -Mg^{2+} in binding buffer, - Mg^{2+} in ATP wash buffer

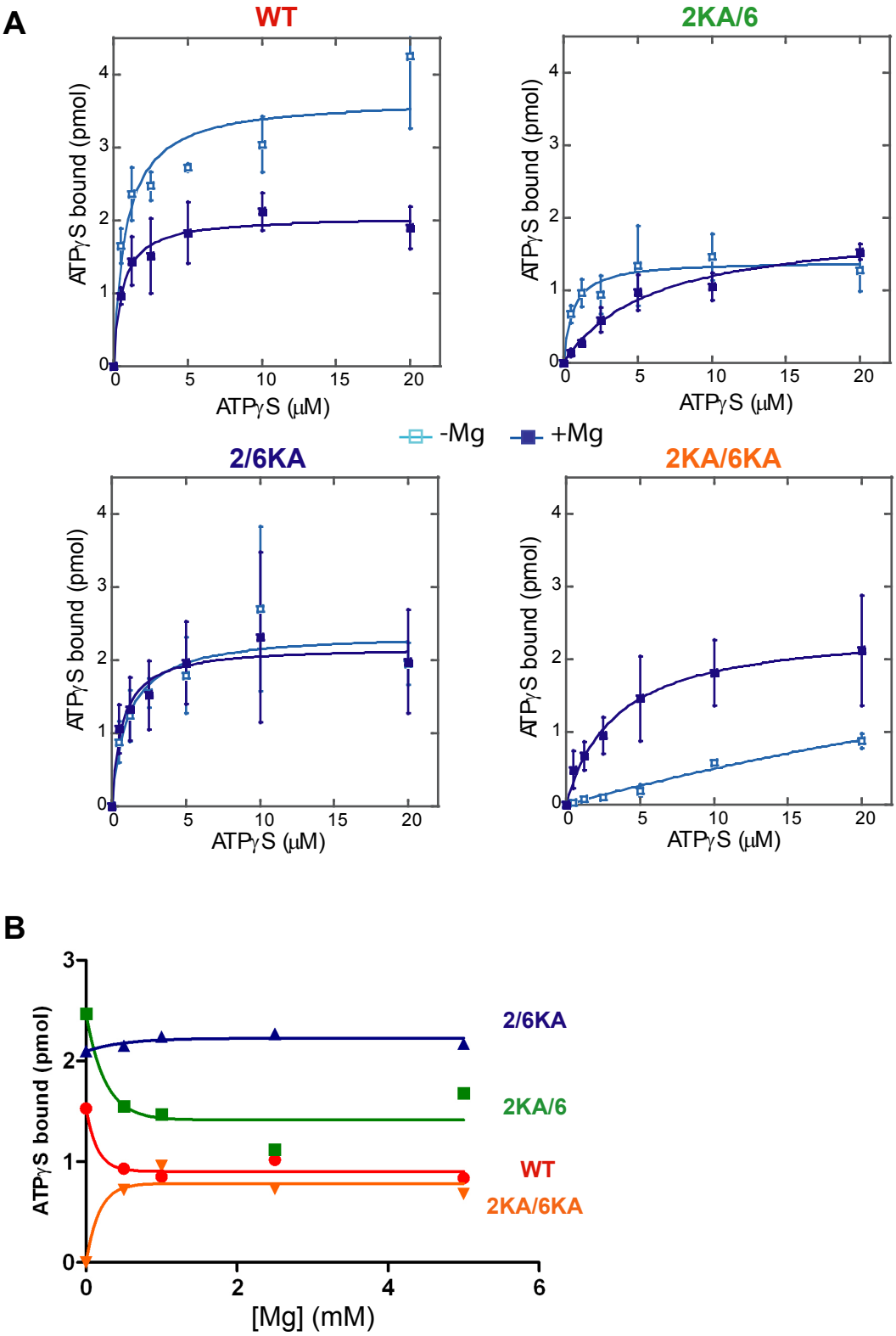

FIGURE S1. The dependence of Mg on WT and K→A heterodimer ATP-γ-S binding. *A,* Filter binding analysis of ATP-γ-S binding to WT or K→A mutant heterodimers (85 nM) with increasing concentrations of ATP-γ-S in the presence or absence of 2.5 mM Mg. *B,* Filter binding analysis of ATP-γ-S binding to WT or K→A mutant heterodimers (85 nM) and ATP-γ-S (5 μM) with various concentrations of Mg. Data points represent an average of at least three experiments with the standard of deviation.

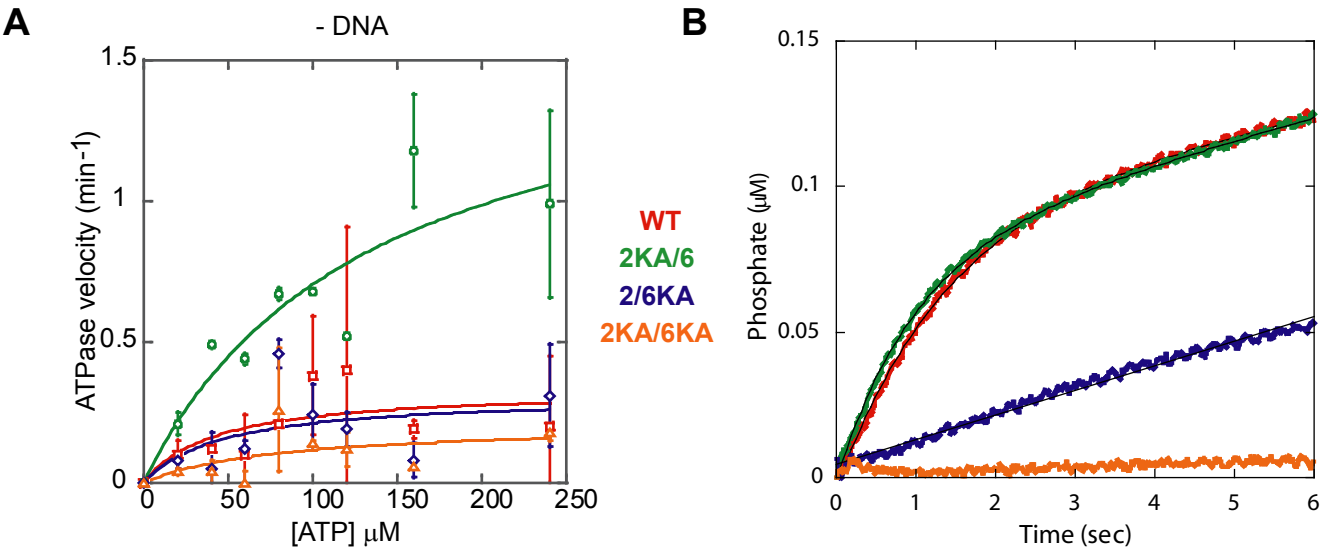

FIGURE S2. The steady-state ATPase activity of hMSH2-hMSH6 in the absence of DNA. *A,* Steady-state ATP hydrolysis in the absence of DNA was performed using WT, 2KA/6, 2/6KA or 2KA/6KA heterodimers (200 nM) and [γ-^{32}P]-ATP. Following incubation at 37 °C for 30 min, the amount of released [γ-^{32}P] was determined. Data points represent an average of three experiments with standard of deviation and were fit with the Michaelis-Menten equation. *B,* Pre-steady state measure of ATP hydrolysis and Pi release was performed using stopped-flow experiments with WT or K→A mutant heterodimers (0.2 mM) and ATP (400 mM).

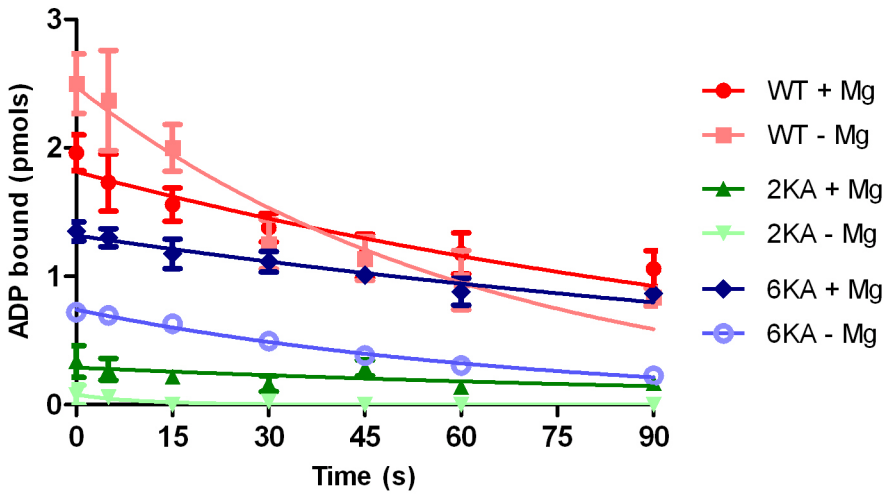

FIGURE S3. Mg inhibits the release of ADP from hMSH2. Nucleotide exchange analysis was performed to examine ADP release following addition of excess ATP without DNA in the presence or absence of Mg (5 mM). Data points represent the average of three independent experiments with standard of deviation.