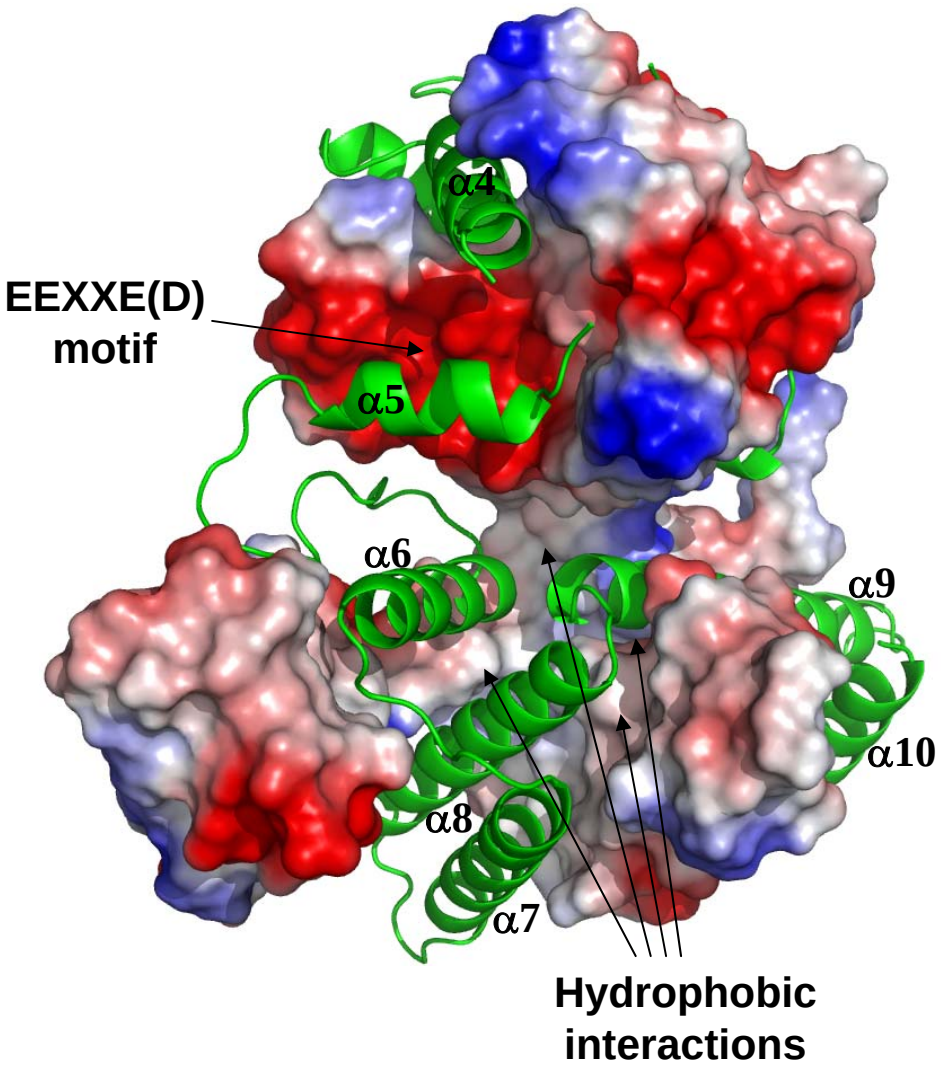
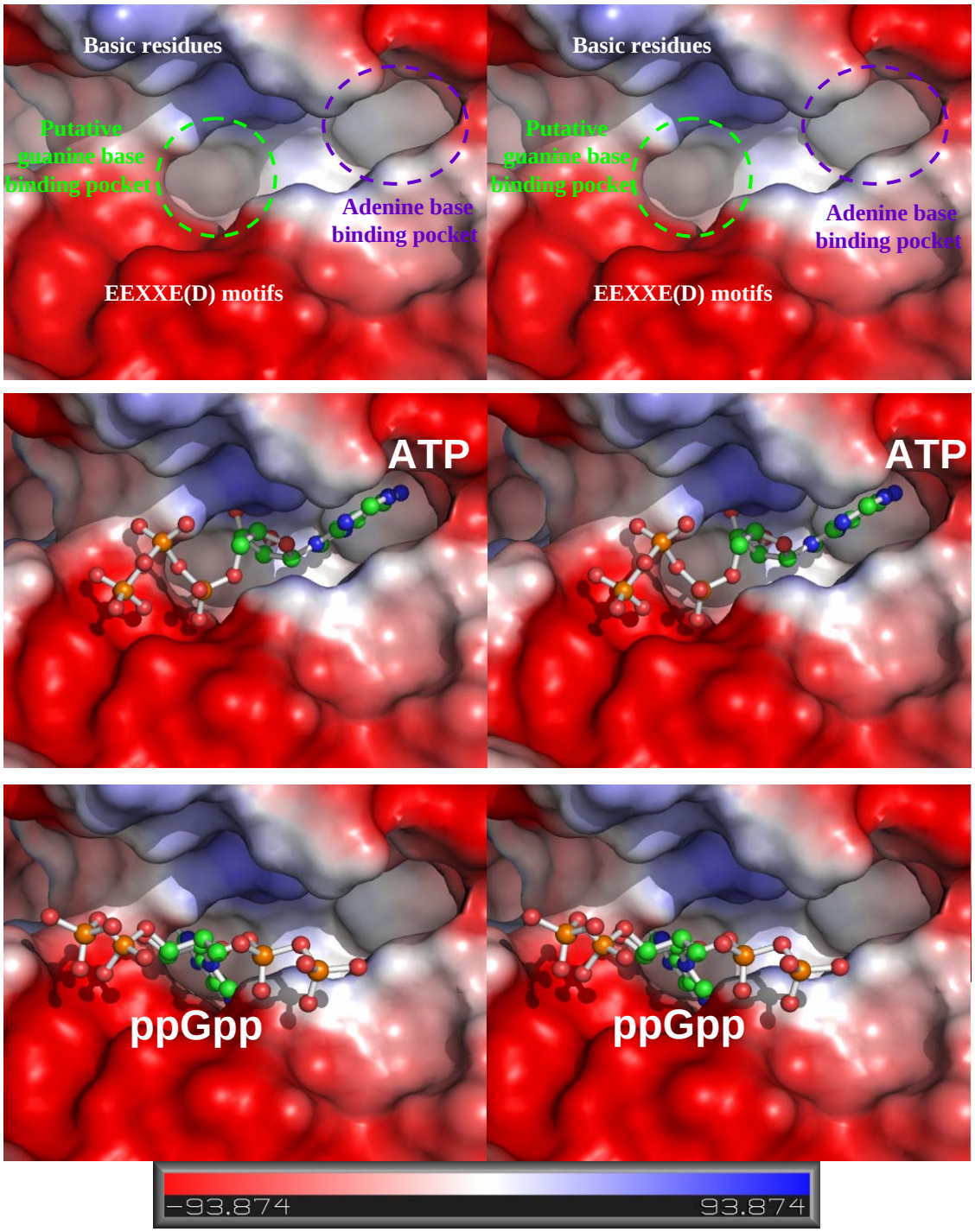
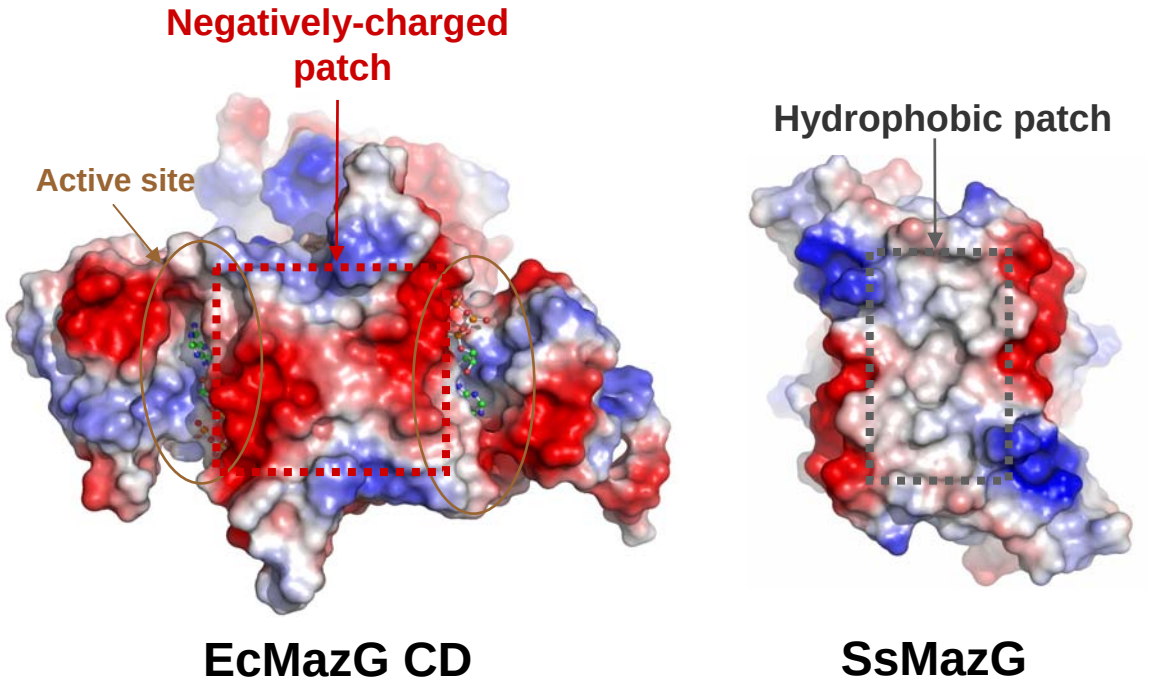


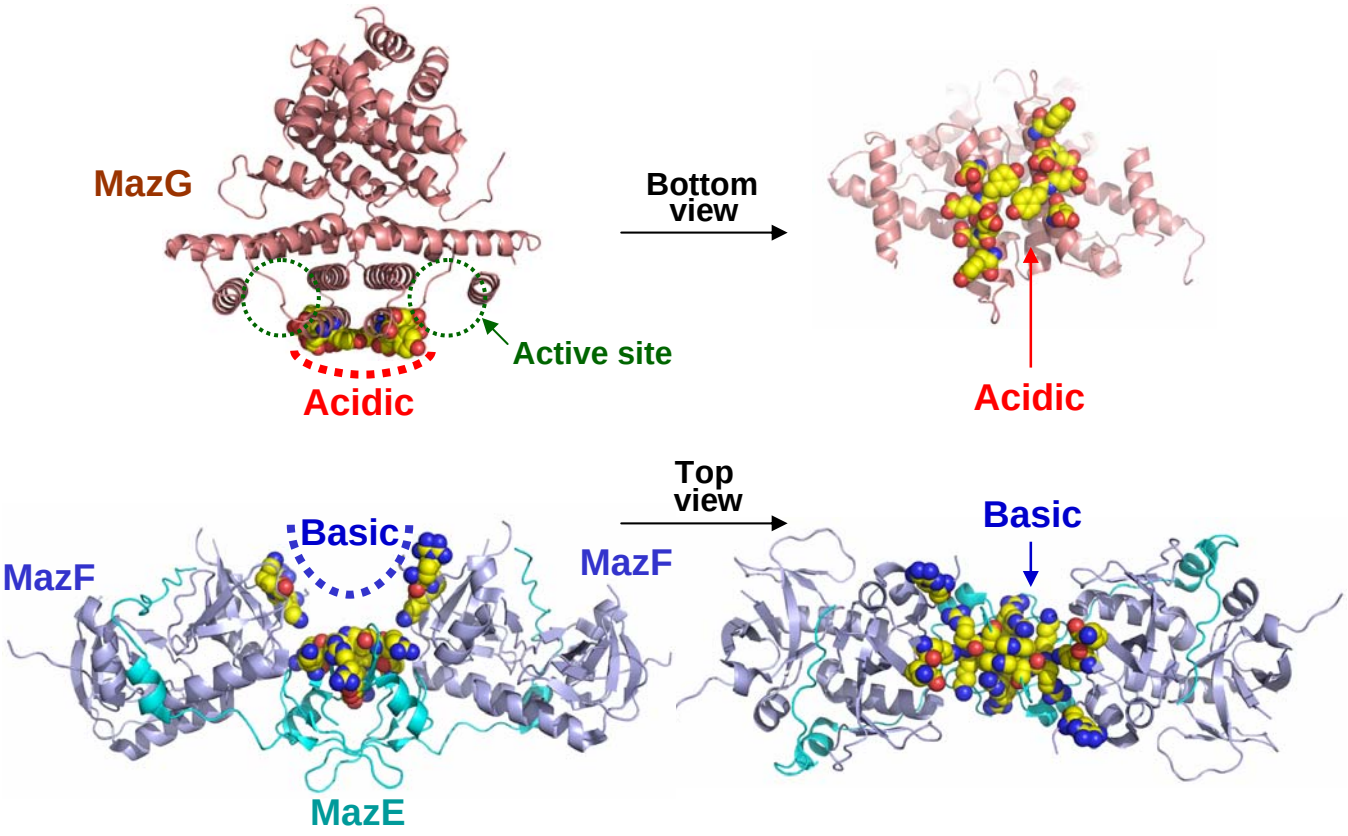
Supplementary Figure 1. Dimerization of EcMazG. One polypeptide is presented by a cartoon diagram, and the other is by a surface-fill model. Secondary structure elements are labeled. Hydrophobic interactions in the 'rigid core' region are identified by arrows.



Supplementary Figure 2. Electrostatic potential stereo presentation of the substrate binding site at the EcMazG CD. A top and a middle figures show the active site without a substrate and with ATP bound. A bottom figure is a modeled structure of EcMazG bound with ppGpp.



Supplementary Figure 3. Surface electro-potential presentations of the bottom sides of the MazG-like proteins. The negatively-charged patch formed at the bottom of the EcMazG CD was compared with hydrophobic patch formed at the same location of SsMazG. EcMazG forms a negatively-charged patch near the active sites in order to prevent a tetramerization. SsMazG and CvMazG form a tetramer by the contact between the hydrophobic patches.



Supplementary Figure 4. Proposed interaction between MazG and MazEF complex. A positively-charged valley constituted by basic residues of MazEF might interacts with a negatively-charged patch on MazG CD, which in turn block the active sites of MazG CD. Acidic and basic surfaces formed on MazG and MazEF are labeled and indicated with red and blue colors, respectively, and the residues involved in these charged-surfaces are presented with sphere-models. MazG, MazE and MazF proteins are shown as cartoon presentations with warm pink, cyan and light blue colors, respectively.

Cloning	Primer sequences	
EcMazG-F	**F**	GCGCGCATATGAATCAAATCGACCGTTTGCTCAC
	R	GCGCGCTCGAGTTAGAGATCAATTTCCTGCCGTTTTAC
EcMazG-ND	**F**	GCGCGCATATGAATCAAATCGACCGTTTGCTCAC
	R	GCGCGCTCGAGTTACGCTTTCTGCGCGCGCTCTTCG
EcMazG-CD	**F**	GCGCGCATATGAGTTTACCGGCTTTAATGCGTGCG
	R	GCGCGCTCGAGTTAGAGATCAATTTCCTGCCGTTTTAC

Mutation	Primer sequences	
R95A	**F**	CATGCGGATGGGCACGCTCTAATTTATC
	R	GATAAATTAGAGCGTGCCCATCCGCATG
K118A	**F**	CGCTCTTCGGTTGCGATTTGCTCCCAAC
	R	GTTGGGAGCAAATCGCAACCGAAGAGCG
K168A	**F**	CCGGTAGTCGATGCAGTCTACGAAGAG
	R	CTCTTCGTAGACTGCATCGACTACCGG
E171A	**F**	CGATAAAGTCTACGCAGAGATCGACGAG
	R	CTCGTCGATCTCTGCGTAGACTTTATCG
E172A	**F**	GATAAAGTCTACGAAGAGATCGACGAGGTG
	R	CACCTCGTCGATCGCTTCGTAGACTTTATC
E175A	**F**	GAAGAGATCGACGCGGTGATGTACGAAG
	R	CTTCGTACATCACCGCGTCGATCTCTTC
K189A	**F**	GTCGACCAGGCTGCACTGGAGGAGGAAATG
	R	CATTTCCTCCTCCAGTGCAGCCTGGTCGAC
E192A	**F**	GCTAAACTGGAGGCGGAAATGGGGGAC
	R	GTCCCCCATTTCCGCCTCCAGTTTAGC
E193A	**F**	CTAAACTGGAGGAGGCAATGGGGGACCTG
	R	CAGGTCCCCCATTGCCTCCTCCAGTTTAG
E196A	**F**	GAGGAAATGGGGGCCCTGCTGTTTGCC
	R	GGCAAACAGCAGGGCCCCCATTTCCTC
K222A	**F**	CAAAAAGCGAACGAAGCATTCGAGCGTCG
	R	CGACGCTCGAATGCTTCGTTCGCTTTTTG
R226A	**F**	CCACTTCGCGAAAAGCACGCTCGAATTTTTC
	R	GAAAAATTCGAGCGTGCTTTTCGCGAAGTGG
W253A	**F**	CAATGGAAGAAGTCGCGCAACAGGTAAAAC
	R	GTTTTACCTGTTGCGCGACTTCTTCCATTG
K257A	**F**	CAATTTCCTGCCGTGCTACCTGTTGCCAG
	R	CTGGCAACAGGTAGCACGGCAGGAAATTG

Supplementary Table 1. Primer sequences used in the study. Primer sequences used for subcloning and mutagenesis are presented. EcMazG-F, EcMazG-ND and EcMazG-CD represent full-length, N- and C-terminal domains, respectively. F and R are abbreviations of forward and reverse primers, respectively. Restriction enzyme sites are underlined.