SUPPLEMENTAL DATA

Metallo-GTPase HypB from *Helicobacter pylori* and Its Interaction with Nickel Chaperone Protein HypA

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Table S1 Primers used for PCR reactions. Restriction sites are highlighted in bold.

Name	Sequence
WT-HypB (forward)	5' GGAATTC**CATATG**AGCGAACAACGACAAGAA 3'
WT-HypB (reverse)	5' CG**GAATTC**TTAAAACGAATGCGTGGACTG 3'
HypBΔ24N (forward)	5' GGAATTC**CATATG**AAGATTTTGAGTAAGAACGACATTA 3'
HypB(M186L, F190V) (forward)	5' AAAGCGGATCTGGTTGAGGTGGTTAATTTCAG 3'
HypB(M186L, F190V) (reverse)	5' ACTGATAATCACCGCATCCGCG 3'
HypB(K168A) (forward)	5' ATGATAAGGTGCTAGCATACCCTACGATGTTCA 3'
HypB(K168A) (reverse)	5' CGCCCTCTGGAACGGAGAGTA 3'
HypB(K18A) (forward)	5' CCCTAATTTGAGTAAAGCAGATGTCAAAATCGTA 3'
HypB(K18A) (reverse)	5' TTATTTTGTAAAGATTCTTGTCGTTGTTCG 3'
HypB(V20A) (forward)	5' TTGAGTAAAAAAGATGCCAAAATCGTAGAAAAG 3'
HypB(V20A) (reverse)	5' ATTAGGGTTATTTTGTAAAGATTCTTGTCGTTGTT 3'
HypB(K21A) (forward)	5' GAGTAAAAAAGATGTCGCAATCGTAGAAAAGATTTT 3'
HypB(K21A) (reverse)	5' AAATTAGGGTTATTTTGTAAAGATTCTTGTCGTT 3'
HypB(I22A) (forward)	5' AAAAAGATGTCAAAGCCGTAGAAAAGATTTTG 3'
HypB(I22A) (reverse)	5' TACTCAAATTAGGGTTATTTTGTAAAGATTCTTGT 3'

Preparation of metal-bound HypB

To prepare Zn^{2+}- or Ni^{2+}-bound HypB proteins, 2 molar equivalents of Zn^{2+} (as $ZnCl_2$) or 5 molar equivalents of Ni^{2+} (as Ni_2SO_4) were added into freshly purified HypB in 20 mM HEPES buffer (pH 7.0) containing 100 mM NaCl. After incubation for 1 hour at 4 ^oC, excess metal ions were removed by HiTrap desalting column. The metal content of protein was measured by ICP-MS (Agilent 7500).

Plasmid construction for GFP-fragment reassembly experiment

HypA protein was fused to the N-terminal fragment (residues 1-157) of GFP (N-GFP) by a spacer linker with sequence of GGSGSGSS and cloned into the plasmid pET32a (AmpR). The C-terminal fragment (residues 158-238) of GFP (C-GFP) was fused similarly to HypB protein by a spacer linker with sequence of GTSGGSG. Particularly, a SD sequence (AAGGAG) was added at the 5′ of *hypB* gene and cloned into the pBAD33 plasmid (CmR) for expression (1). The DNA fragments of N-GFP and C-GFP were amplified by PCR reactions using pEGFP plasmid as template, *hypA* and *hypB* genes were amplified from *H. pylori 26695* genomic DNA. The DNA fragments were fused together by bridging PCR (2) using primers listed in Table S2.

Table S2 Primers used for plasmid construction in GFP-fragment reassembly experiment. Restriction sites are highlighted in bold and spacer linkers are shown as lowercase.

For N-GFP	
N-GFP(*Nde*I)-for:	5' GGAATTC**CATATG**GTGAGCAAGGGCG 3'
N-GFP(*Xho*I)-rev:	5' CCG**CTCGAG**TTACTGCTTGTCGGCCATG 3'
For C-GFP	
C-GFP(*EcoR*I)-for:	5' G**GAATTC**ATGAAGAACGGCATCAAGG 3'
C-GFP(*Hind*III)-rev:	5' CCC**AAGCTT**TTACTTGTACAGCTCGTCCATG 3'
For NGFP-HypA	
N-GFP(*Nde*I)-for:	5' GGAATTC**CATATG**GTGAGCAAGGGCG 3'
N-GFP(spacer)-rev:	5' AGccagagccagagccaccCTGCTTGTCGGCCATG 3'
HypA(spacer)-for:	5' gctctggctctggCTCGTCcATGCATGAATACTCGGTCGT 3'
HypA(*Xho*I)-rev:	5' CCG**CTCGAG**TTATTCCGCTAACATTTCTAAAGA 3'
For HypB-CGFP	
HypB(*EcoR*I)-for:	5' G**GAATTC**ATGAGCGAACAACGACAAGA 3'
HypB(spacer)-rev:	5' cgcttccacccGACGTCccAAACGAATGCGTGGACT 3'
C-GFP(spacer)-for:	5' CGTCgggtggaagcggtaagATGAAGAACGGCATCAAGG 3'
C-GFP(*Hind*III)-rev:	5' CCC**AAGCTT**TTACTTGTACAGCTCGTCCATG 3'

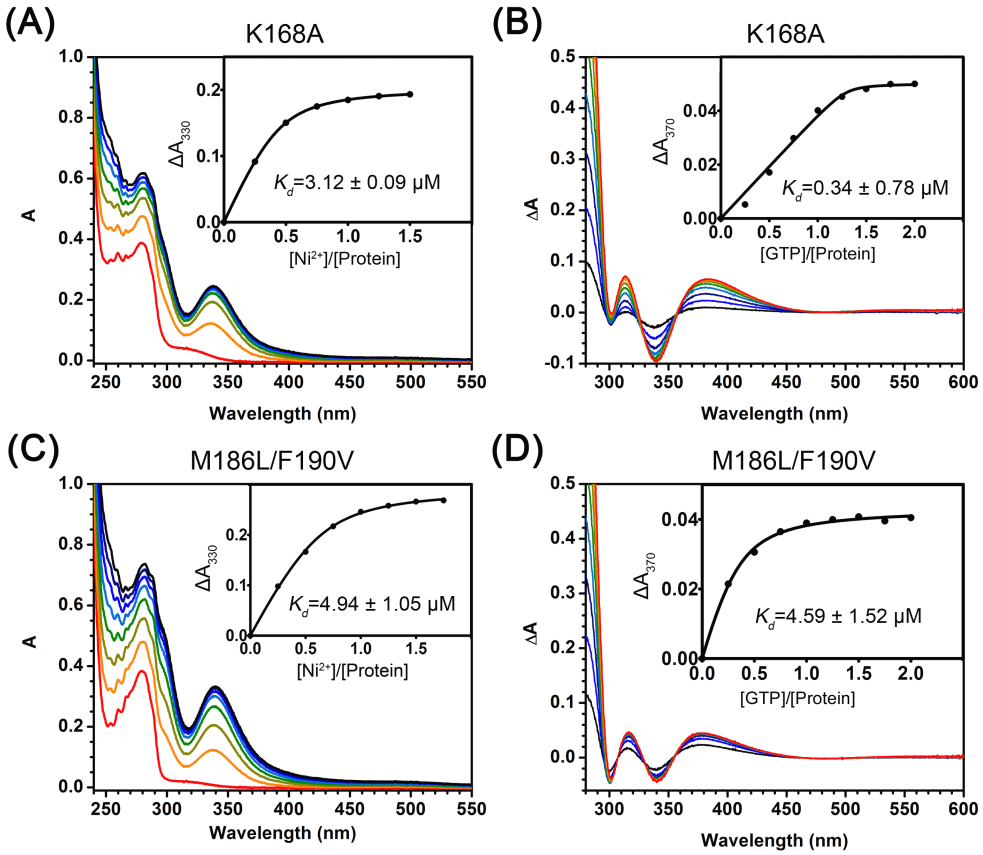

FIGURE S1 Binding of Ni^{2+} and GTP to *Hp*HypB mutants. UV-vis spectra of 50 μM mutant proteins ***(A)*** HypB(K168A) and ***(C)*** HypB(M186L/F190V) in the absence and presence of different molar equivalents of Ni^{2+} (as 10 mM $NiSO_4$) in 20 mM Tris-HCl buffer, pH 7.4 containing 100 mM NaCl, 200 μM TCEP. Difference UV-vis spectra of ***(B)*** Ni^{2+}-HypB(K168A) and ***(D)*** Ni^{2+}-HypB(M186L/F190V) with increasing amounts of GTP. The titration curves were fitted to Ryan-Weber nonlinear equation to determine the dissociation constants (K_d).

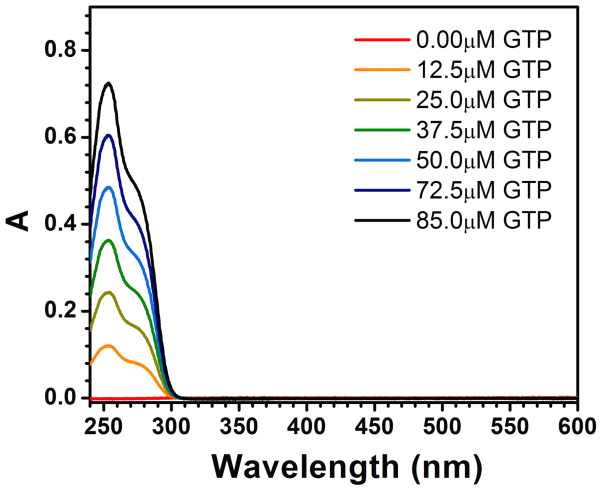

FIGURE S2 Different UV-vis spectra of Ni^{2+} solution upon titration of GTP. The spectra were recorded for approximate 50 μM $NiSO_4$ in 20 mM Tris-HCl buffer containing 100 mM NaCl, 200 μM TCEP, and 1 mM $MgSO_4$ at pH 7.4 with stepwise addition of GTP.

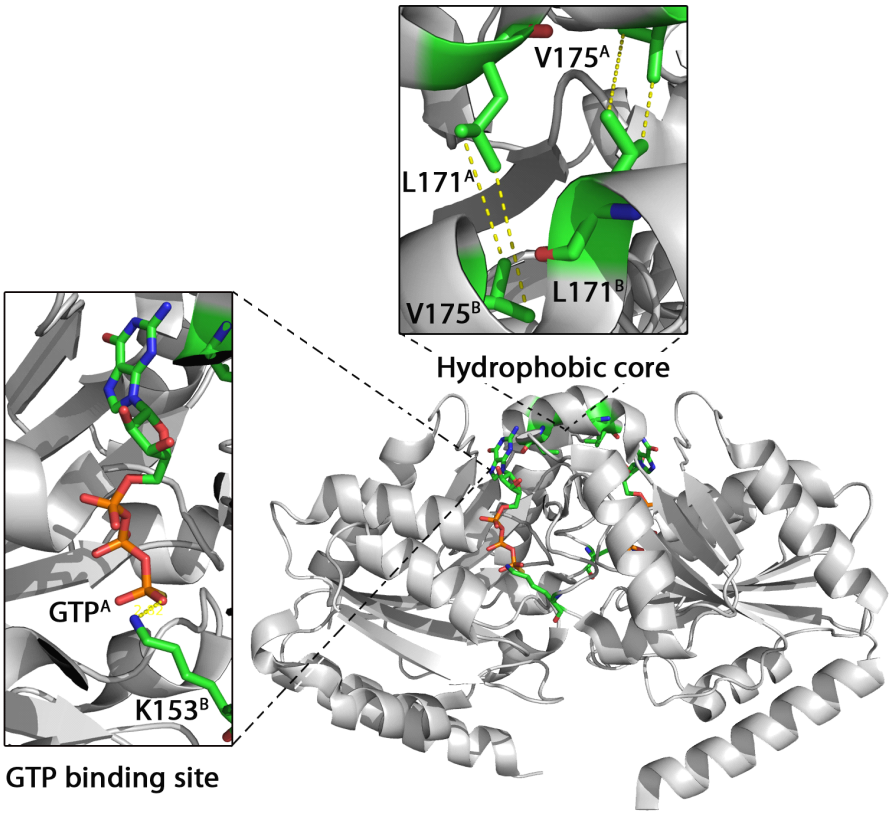

FIGURE S3 Crystal structure of *Mj*HypB (PDB ID: 2HF9). The GTP binding site containing the bound GTP molecule and conserved K153, and the hydrophobic core region that contains L171 and V175 are highlighted.

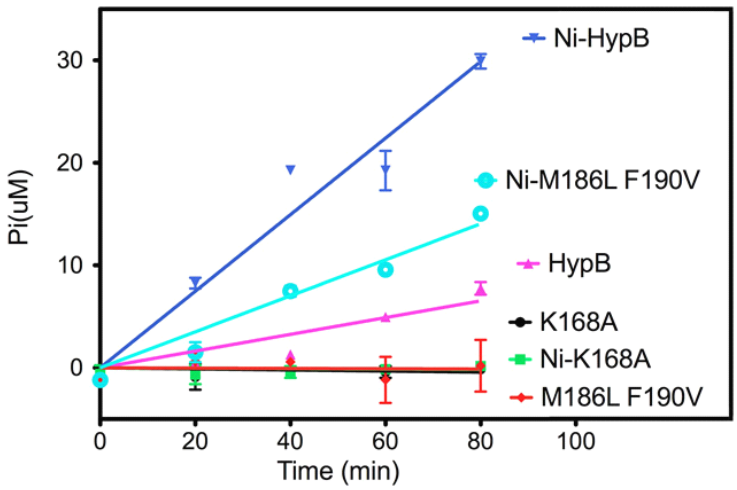

FIGURE S4 GTPase activities of HypB and its mutants determined by Malachite Green method. The GTP hydrolysis rates for all protein samples are linear within 80 mins.

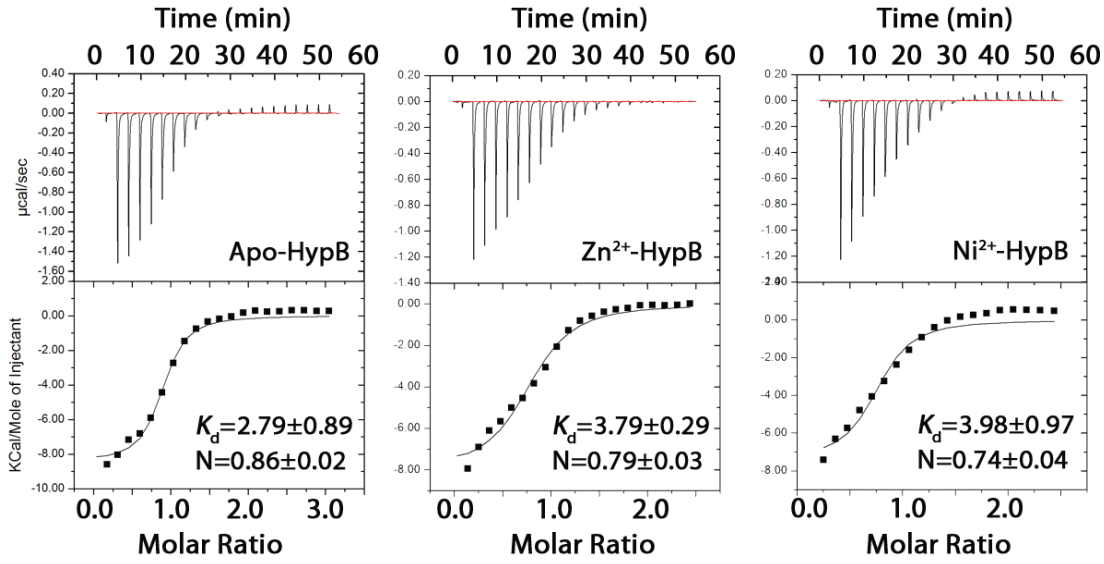

FIGURE S5 Determination of binding affinities of GTP to HypB proteins by isothermal titration calorimetry. The binding affinities of GTP to apo-, Zn^{2+} and Ni^{2+}-HypB are similar.

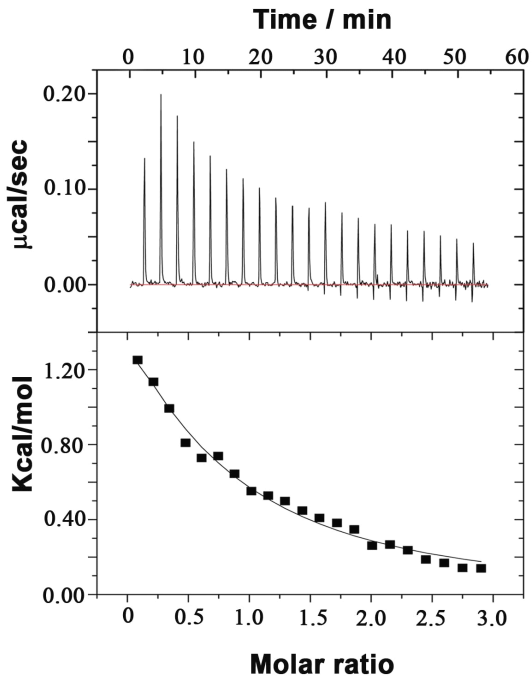

FIGURE S6 Determination of binding affinities of HypA to HypB by isothermal titration calorimetry. About 1.1 mM HypA in 20 mM HEPES buffer containing 100 mM NaCl, 2 mM TCEP, pH 7.0 was titrated into 0.1 mM apo-HypB in the same buffer and the dissociation constant (K_d)was fitted to be 57.9±8.5 µM.

Table S3 MALDI-TOF MS/MS analysis of trypsin digested protein bands from SDS-PAGE.

Sample	Observed m/z	Predicted m/z	Predicted sequence[a]	Assignment
Gel-band (~13 kDa)	828.4812	827.4739	VVVGIGER	HypA(29-36)
	1274.6421	1273.6348	NVIITQGNEMR	HypA(98-108)
	1290.6326	1289.6253	NVIITQGNEMR.L + Oxidation (M)	HypA(98-108)
	2149.0220	2148.0147	SLFVSAFETFREESLVCK	HypA(42-59)
	2681.2380	2680.2307	SAMDKSLFVSAFETFREESLVCK	HypA(37-59)
Gel-band (~27 kDa)	1227.5720	1226.5647	ADMVEVFNFR	HypB(184-193)
	1482.7592	1481.7519	SLEDFKNFLLEK	HypB(220-231)
	1685.7875	1684.7802	EGLYVLNFMSSPGSGK	HypB(44-59)
	2090.0212	2089.0139	YLKEGLYVLNFMSSPGSGK	HypB(41-59)
	3091.4070	3090.3997	TTMLENLADFKDFKFCVVEGDLQTNR	HypB(60-85)
Gel-band (~40 kDa)	828.4967	827.4894	VVVGIGER	HypA(29-36)
	1274.6780	1273.6707	NVIITQGNEMR	HypA(98-108)
	1227.6097	1226.6024	ADMVEVFNFR	HypB(184-193)
	839.4700	838.4627	NA[b]	NA
	800.4542	799.4469	NA	NA

a. MS data were collected on an ABI 4800 MALDI TOF/TOF analyzer with QuanTIS precursor selector that allows the detection and identification of peptides at high resolution. Some of the digested peptides were subjected to MS/MS to confirm the peptide sequence and results were analyzed using MASCOT sequence query server.

b. NA: no matched peptide.

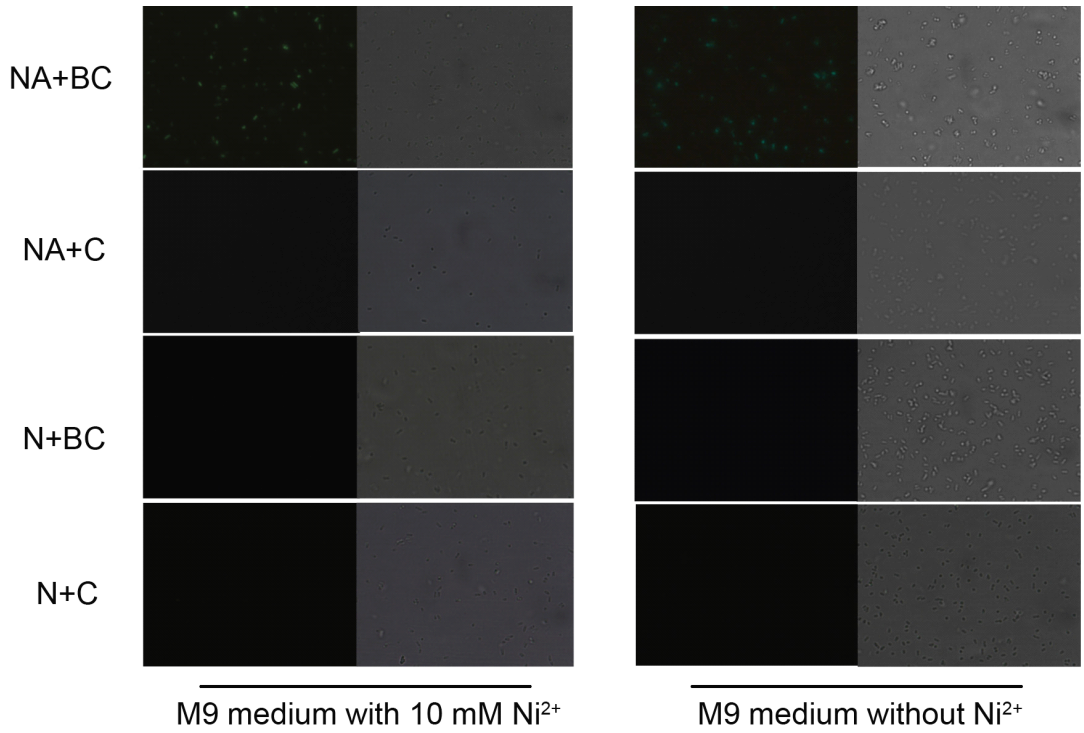

FIGURE S7 The effect of Ni^{2+} on the intracellular fluorescence. Different combinations of fusion proteins are co-transformed into *E. coli* cells and co-expressed by addition of 100 μM IPTG and 0.015% arabinose in M9 minimal medium with or without 10 mM Ni^{2+}. Phase contrast and fluorescence images of the cells were recorded. *E. coli* cells that co-expressed NGFP-HypA and HypB-CGFP proteins (NA+BC) yield green fluorescence, indicating that interaction between HypA and HypB was independent on the availability of Ni^{2+}.

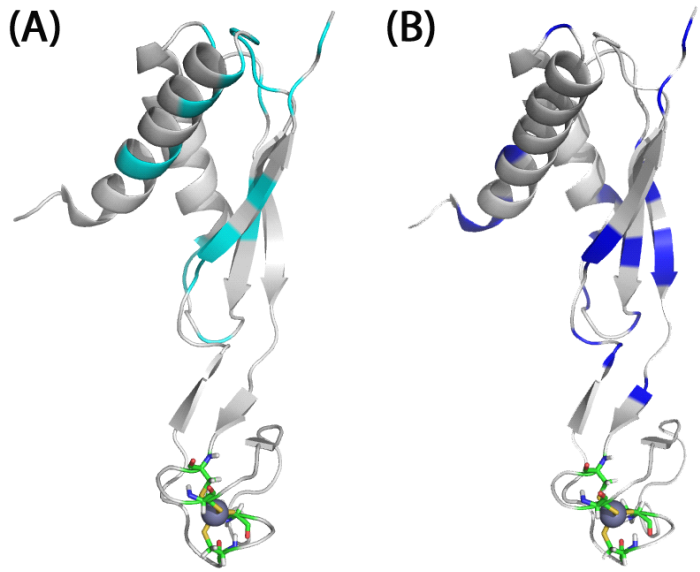

FIGURE S8 Mapping the residues experiencing large chemical shift perturbations upon binding of (A) HypB and (B) Ni^{2+} onto the structure of HypA, indicative of a similar pattern of location of those perturbed residues.

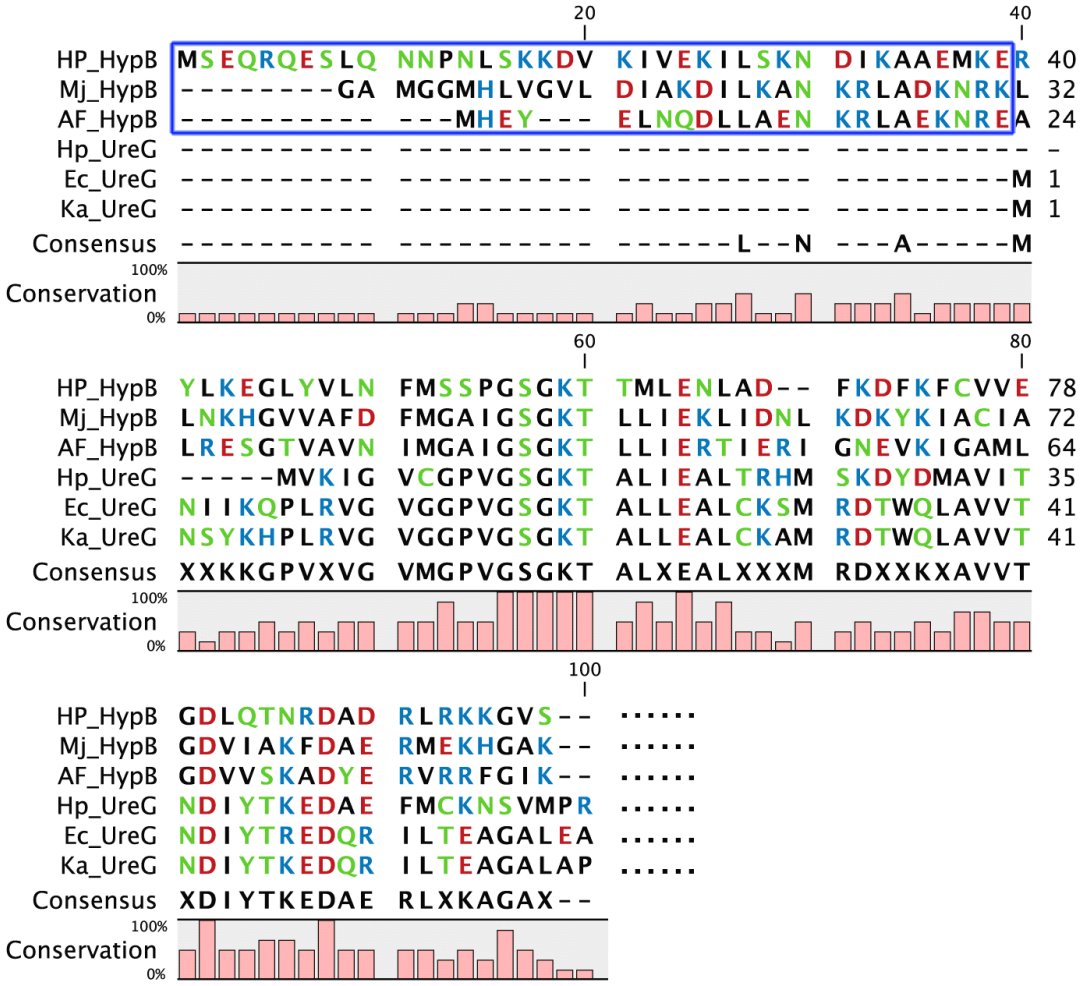

FIGURE S9 Sequence alignments of HypB and UreG proteins. The alignment is performed with HypB homologues of *Helicobacter pylori* 26695 (Hp_HypB), *Methanococcus jannaschii* (Mj_HypB), *Archaeoglobus fulgidus* (Af_HypB) and UreG homologues of *Helicobacter pylori* 26695 (Hp_UreG), *Escherichia coli* O157:H7 (Ec_UreG), *Klebsiella aerogenes* (Ka_UreG). The unique N-terminal regions of HypB homologues are highlighted in blue box.

References

1. Guzman, L. M., Belin, D., Carson, M. J., Beckwith, J. (1995) *J. Bacteriol.* **177**, 4121-4130.
2. Tao, A. L., He, S. H. (2004) *World J. Gastroenterol.* **10**, 2103-2108.