Supplemental Information for:

"The Effect of Metal on the Biochemical Properties of the *Helicobacter pylori* HypB, a Maturation Factor of [NiFe]-Hydrogenase and Urease"

A. M. Sydor, J. Liu, and D. B. Zamble

Table S1: PCR primers used for cloning and mutagenesis.[a]

Primer Name	Primer Sequence
HpHypB forward	5'AAGGAAC**CATATG**AGCGAACAACGACAAG3'
HpHypB reverse	5'TCGCTA**CTCGAG**TTAAAACGAATGCGTGG3'
C106A, H107A forward	5'CACCACCGGCGAAGCAgcCgcTTTGGAAGCGAGCATG3'
C106A, H107A reverse	5'CATGCTCGCTTCCAAAgcGgcTGCTTCGCCGGTGGTG3'

[a] Restriction enzyme sites are shown in bold. Mutations are shown in lowercase.

Table S2: Summary of gel filtration chromatography results of WT and mutant *Hp*HypB with added metals.[a]

Protein	Added Metal	Elution Volume (mL)	Calculated MW (kDa)	Relative Peak Area[b]	Assignment
WT *Hp*HypB	**Apo**	16.9 ± 0.2	28.1 ± 2.6	100	Monomer
	0.5 eq. Ni(II)	15.9 ± 0.1	44.7 ± 1.6	76	Dimer
		17.2 ± 0.1	23.0 ± 0.7	24	Monomer
	1 eq. Ni(II)	15.7 ± 0.1	50.9 ± 1.9	100	Dimer
	1 eq. Zn(II)	15.6 ± 0.1	52.7 ± 2.7	3.5	Dimer
		17.0 ± 0.1	25.8 ± 1.0	96.5	Monomer
	1 eq. Zn(II) + 1 eq. Ni(II)	15.86 ± 0.01	46.2 ± 0.2	5	Dimer
		17.34 ± 0.02	21.9 ± 0.2	95	Monomer
C106A, H107A *Hp*HypB	**Apo**	17.2 ± 0.1	23.8 ± 0.8	100	Monomer
	1 eq. Ni(II)	17.1 ± 0.1	24.5 ± 0.8	100	Monomer
	1 eq. Zn(II)	17.11 ± 0.01	24.6 ± 0.1	100	Monomer

[a]Errors are standard deviations of at least two replicates. The expected molecular weights are: WT *Hp*HypB monomer: 27.2 kDa; WT *Hp*HypB dimer: 54.4 kDa; C106A, H107A *Hp*HypB monomer: 27.1 kDa.
[b]The relative peak areas varied by <3.5 % of the total peak area.

Table S3: Summary of gel filtration chromatography results of WT *Hp*HypB with added nucleotides.[a]

Added ligand	Elution Volume (mL)	Calculated MW (kDa)	Relative Peak Area[b]	Assignment
5 eq. GTP	15.9 ± 0.2	45.4 ± 4.0	8	Dimer
	17.2 ± 0.3	24.0 ± 3.2	92	Monomer
5 eq. GDP	16.0 ± 0.1	43.9 ± 1.7	9.5	Dimer
	17.4 ± 0.1	21.3 ± 0.7	90.5	Monomer
1 eq. Ni(II) + 5 eq. GDP	15.8 ± 0.2	48.0 ± 4.4	81.5	Dimer
	17.21 ± 0.04	23.4 ± 0.4	18.5	Monomer
1 eq. Ni(II) + 5 eq. GTP	16.03 ± 0.04	42.4 ± 8.0	84	Dimer
	17.29 ± 0.03	22.4 ± 0.3	16	Monomer
5 eq. GTP + 400 µM GTP in MP	16.8 ± 0.1	58.4 ± 3.5	31.5	Dimer
	18.34 ± 0.04	27.6 ± 0.6	68.5	Monomer
5 eq. GDP + 400 µM GDP in MP	16.99 ± 0.01	54.2 ± 0.4	27	Dimer
	18.3 ± 0.1	28.1 ± 2.0	73	Monomer
5 eq. GTP + 400 µM GTP in MP + 1 eq. Zn(II)	18.33 ± 0.04	27.9 ± 0.5	100	Monomer
5 eq. GDP + 400 µM GDP in MP + 1 eq. Zn(II)	18.225 ± 0.007	29.3 ± 0.1	100	Monomer

[a]Errors are standard deviations of at least two replicates. The expected molecular weights are: WT *Hp*HypB monomer; 27.2 kDa; WT *Hp*HypB dimer; 54.4 kDa. MP indicates that the nucleotide was included in the mobile phase.
[b]The relative peak areas varied by <3.5 % of the total peak area.

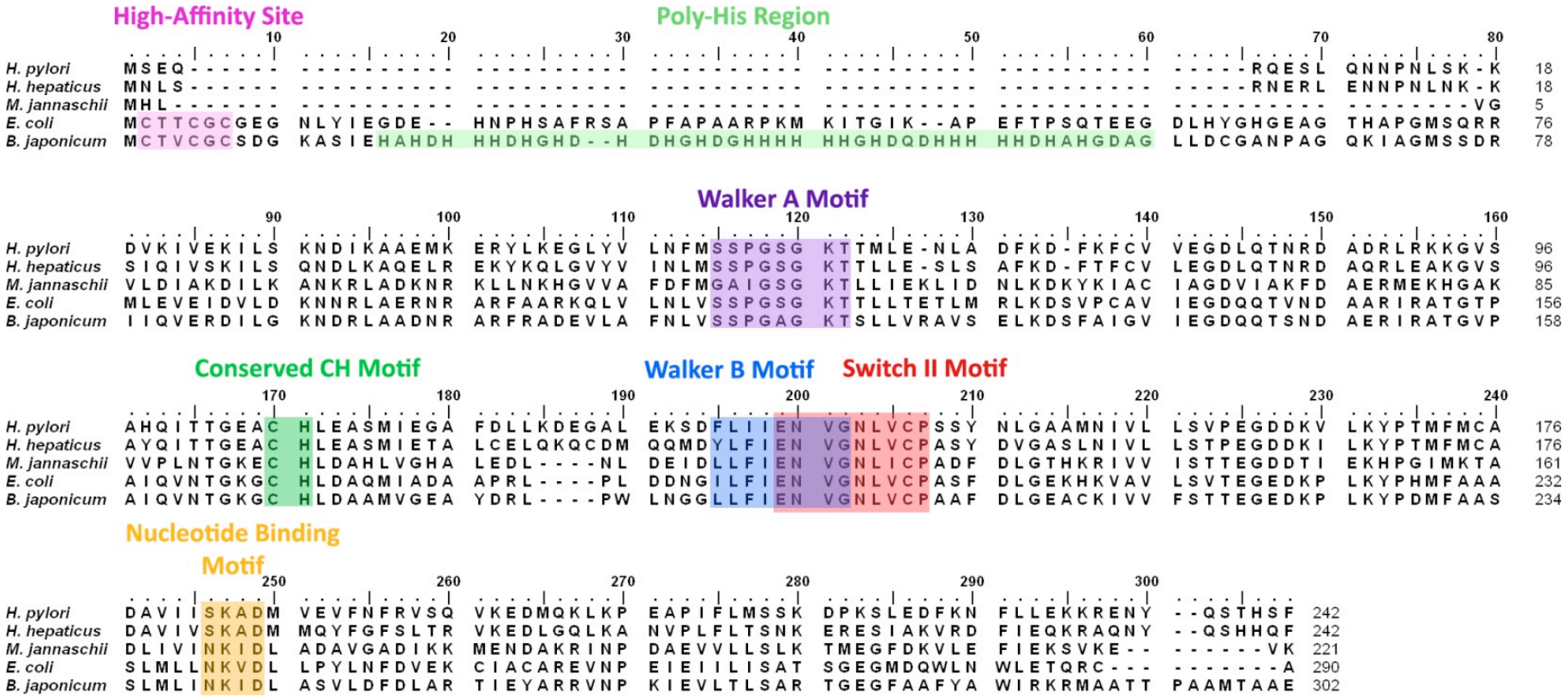

Figure S1. Amino acid alignment of HpHypB with homologs. The specific strains are as follows: *Escherichia coli* K12 substr. MG1655, *Helicobacter pylori* 26695, *Helicobacter hepatics* ATCC 51449, *Bradyrhizobium japonicum* USDA110, and *Methanocaldococcus jannaschii* DSM 2661. All sequences were retrieved from the NCBI database and aligned using ClustalW (1).

REFERENCES

1. **Higgins, D. G., and Sharp, P. M.** 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene 73:237-244.