Electronic Supporting Information for

Interaction of SlyD with HypB of *Helicobacter pylori* facilitates nickel trafficking

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Materials and Methods

PCR amplification was performed by using Phusion® High-Fidelity DNA Polymerase (Finnzymes). All restriction endonucleases were purchased from New England BioLabs. PCR products and enzyme digested products were purified by using illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare).

Expression and purification of proteins

The expression plasmids pET32-HpSlyD (for *Hp*SlyD) and pET32-HpSlyDΔC (for the C-terminus-truncated *Hp*SlyD, *Hp*SlyDΔC) are from previous work.[1] The expression plasmids pET28-HypB (for *Hp*HypB) and pET28-HypBΔ24N (for the N-terminal 24-residue-deleted *Hp*HypB, *Hp*HypBΔ24N) was also constructed as reported previously.[2] The expression plasmid pGBHIS-HypA (for *Hp*HypA) is from previous work.[3]

Table S1 Primers used

Name	Sequence (5'-3')	Enzyme
HA-SlyD-up	GGAATTCCATATGTACCCATACGACGTACCAGATTACGCTATGCAAAACCATGATTTAGAG	*Nde*I
Myc-HypB-up	GGAATTCCATATGGAGCAGAAGCTGATCTCAGAGGAGGACCTGATGAGCGAACAACGACAAGAA	*Nde*I
SlyD(spacer)-for	GCTCTGGCTCTGGCTCGTCCATGCAAAACCATGATTTAGAG	
SlyD-down(XhoI)	CCGCTCGAGCTACCCATGCGAACATGAGCA	*Xho*I
FKBP-for	TTAGCGTTTCGTTTCAAGGTTT	
FKBP-rev	CCCATAAGCTTCCTCTGGG	

HA-tag (YPYDVPDYA) was fused to the N-terminus of *Hp*SlyD as HA-*Hp*SlyD. Myc-tag (EQKLISEEDL) was fused to the N-terminus of *Hp*HypB as Myc-*Hp*HypB. HA-*Hp*SlyD gene fragment was amplified by using the primer pair HA-SlyD-up/HpSlyD-rev[1], generating pET32-HA-HpSlyD, and Myc-*Hp*HypB gene fragment was amplified by using the primer pair Myc-HypB-up/WT-HypB (reverse)[2], generating pET28-Myc-HpHypB.

Two fragments flanking the IF domain were amplified by using the primer pairs HpSlyD-for/FKBP-rev and FKBP-for/HpSlyD-rev. The two obtained and purified fragments were then mixed together and the *Hp slyD-FKBP* fragment was amplified using *Hp*SlyD-for/*Hp*SlyD-rev and cloned into pET-32a(+) as previously,[1] generating pET32-FKBP. *Hp*SlyD-FKBP is thus the fusion of 1-74 and 135-185 of *Hp*SlyD.

pET28-HypB, pET28-HypBΔ24N and pET28-Myc-HypB were transformed into *E. coli* BL21(DE3), and pET32-HA-SlyD and pET32-FKBP were transformed into *E. coli* KMl603 [BL21(DE3) ΔslyD::kan (a gift from A.R. Davidson, University of Toronto).
*Hp*HypB and its variants were expressed and purified following a previous description.[2] HA-*Hp*SlyD and *Hp*SlyD-FKBP was prepared as the wild-type *Hp*SlyD previously described.[1]

All proteins were treated with 1 mM TCEP and 1 mM EDTA overnight at 4 °C and desalted into corresponding buffer. The apo-state of each protein was examined by determining metal contents with ICP-MS. Protein concentration was determined by using BCA Protein Assay Kit (Novagen).

NMR titration of *Hp*SlyD with *Hp*HypB

The NMR titration was done similarly to the previous description.[1] Proteins were prepared in the NMR buffer (20 mM BisTris-HCl buffer, pH 6.8, containing 100 mM NaCl, 10% (v/v) D_2O, 0.02% NaN_3). About 0.3 mM of ^{15}N-labeled *Hp*SlyDΔC was used for the titration with unlabeled *Hp*HypB up to 1:1 molar ratio ([protein]/[*Hp*SlyDΔC]).

Cross-linking assays

Purified proteins were firstly treated by 1 mM TCEP and 10 mM EDTA overnight at 4 °C and desalted into 10 mM sodium phosphate buffer (pH 6.5). For cross-linking assays, 30 μM *Hp*SlyD was incubated with other interacting partner proteins (30 μM), e.g. *Hp*HypB and their variants overnight with mixture at 4 °C in the same buffer. EDC (1-ethyl-3-[3-

dimethylaminopropyl]carbodiimide hydrochloride, Sigma Aldrich) was added into the reaction system to a final concentration of 10 mM and the samples were further incubated for 2 hrs at 25 °C before the cross-linking was quenched by adding final 50 mM Tris-HCl pH 8.0. For analyzing the assays, samples were subjected on polyacrylamide gels for SDS-PAGE and the bands were visualized by Coomassie Brilliant Blue.

Western blot

Cross-linked samples were separated duplicately in a 5-15 % gradient SDS-PAGE gel and were transferred onto an Amersham™ Hybond™-P membrane (GE Healthcare) in transfer buffer (25 mM Tris, 192 mM glycine and 20% methanol) for 1 h at 90 V. The membrane was blocked for 2 h at room temperature in TBST+BSA buffer (Tris buffered saline, 0.1% Tween 20, 5% BSA). The membrane was cut into two halves and each half was incubated with 1:1000-diluted primary monoclonal anti-HA (HA-7) and anti-Myc (9E10) antibodies produced in mouse (Sigma), respectively, in TBST+BSA buffer for 1 h at room temperature. The membranes were washed 5 times for 10 minutes with TBST buffer, and then incubated for 1 h with 1:5000-diluted ECL™ Mouse IgG, HRP-Linked Whole Ab (from sheep) (GE Healthcare) in TBST+BSA buffer. After washed 5 times for 10 minutes with TBST buffer, the membranes were revealed with Amersham™ ECL™ Western blotting reagent (GE Healthcare).

GFP-fragment reassembly

In order to visualize the interaction between *Hp*SlyD and *Hp*HypB, a GFP fragment reassembly assay was performed referring to a previously described method.[4] An enhanced GFP variant EGPF (λ_{ex}= 488 nm, λ_{em}= 507 nm, Clontech) was chosen for the assay. The cutting point for the N-terminal and C-terminal fragments of EGFP (N-EGFP and C-EGFP) was referred to the previous description.[2,4] The plasmid pEGFP (Clontech) was used as the template for PCR amplification of N-EGFP and C-EGFP fragments.

Previously constructed plasmids pET32a-NGFP, pBAD33-CGFP, and pBAD33-HypB-linker-CGFP were used in this study, which encode N-EGFP, C-EGFP and C-EGFP-fused *Hp*HypB.[2] The N-EGFP-fused *Hp*SlyD was constructed as follows. N-EGFP fragment was amplified by using the primer pair N-GFP(NdeI)-for/N-GFP(spacer)-rev.[2] *Hp*SlyD fragment was amplified by using the primer pair SlyD(spacer)-for/SlyD-down(XhoI) with the plasmids constructed previously.[1] The fragment for NGFP-SlyD was amplified by overlap PCR by using the forward primer N-

GFP(NdeI)-for and the reverse primer SlyD-down(XhoI). The amplified fragments with correct length were purified and digested with *Nde*I and *Xho*I and inserted into pET-32a(+), generating the plasmids pET32-NGFP-SlyD.

Both bait and prey plasmids in different combination were co-transformed into *E. coli* BL21(DE3) cells for expression of both N-EGFP and C-EGFP fused proteins.

For GFP fragment reassembly assay, BL21(DE3) cells harboring both plasmids were cultured in LB media at 37 °C overnight and then sub-cultured with 1:100 dilution until OD_{600} to 0.6. The expressions of both bait and prey proteins were induced by the addition of final 10 μM IPTG and 0.2% L-(+)-arabinose respectively and cultured further at 25 °C for 2 days. For visualization of reassembled EGFP complex, the above cultured cells were washed by PBS buffer for three times by centrifugation at 5000 *g* at 4 °C. Cells were then diluted to OD_{600} 0.3 and subjected under a Leica DMI3000 B inverted microscope equipped with an I3 filter cube for inspection.

GTPase assay

The determination of GTPase activity was performed by using the Malachite Green Phosphate Assay Kit (Cayman Chemical) based on the method of D'Angelo *et al*. to determine the free phosphate.[5] Series of 50-μl samples containing 2 μM *Hp*HypB (in the GTPase reaction buffer: 20 mM HEPES, 100 mM NaCl, pH 7.0, 5 mM $MgCl_2$, 5 % glycerol) with or without equal molar ratio of partner proteins, and various GTP concentrations between 12.5 and 500 μM were incubated at 37 °C for 1.5 h. The same series of solutions without *Hp*HypB but with the same concentrations of GTP were also incubated as blank controls. After incubation, the samples were quickly plated on a 96-well plate and 5 μl of MG Acid Solution was added to each sample well and the samples were incubated for 10 min at room temperature. Then, 15 μl of MG Blue Solution was added into each well and the samples were further incubated for 20 min before subjected to a microplate reader for determining the absorbance at 620 nm. The amount of phosphate was calculated by using a standard curve prepared by MG Phosphate Standard (1 M). The amount of phosphate released in the reaction was obtained by subtraction of the self-hydrolysis of GTP. The kinetic parameters were obtained by non-linear fitting based on the Michaelis-Menten equation. Metal-bound proteins were prepared by incubating appropriate amounts of metals with apo-proteins overnight and then desalted into the GTPase reaction buffer by using HiTrap Desalting column (GE Healthcare). The stoichiometry of bound metals for each protein sample was determined by ICP-MS.

Metal transfer assay using analytical size-exclusion chromatography

Analytical size-exclusion chromatography (SEC) was performed on an ÄKTAFPLC system (280 nm UV detection) with a Superdex 75 10/300 GL column (GE Lifescience). Proteins were pre-treated with 1 mM TCEP and loaded with excess metal ions (for metal-bound samples) and desalted into the HEPES buffer (20 mM HEPES, 100 mM NaCl, 500 µM TCEP, pH 7.4). Each sample contained 100 µM of *Hp*HypB (apo- or metal-bound form) unless otherwise noted. Different combination of *Hp*HypB and *Hp*SlyD were mixed and incubated at 4 °C overnight. Around 100 µl of sample were loaded onto the column and eluted by the same buffer.

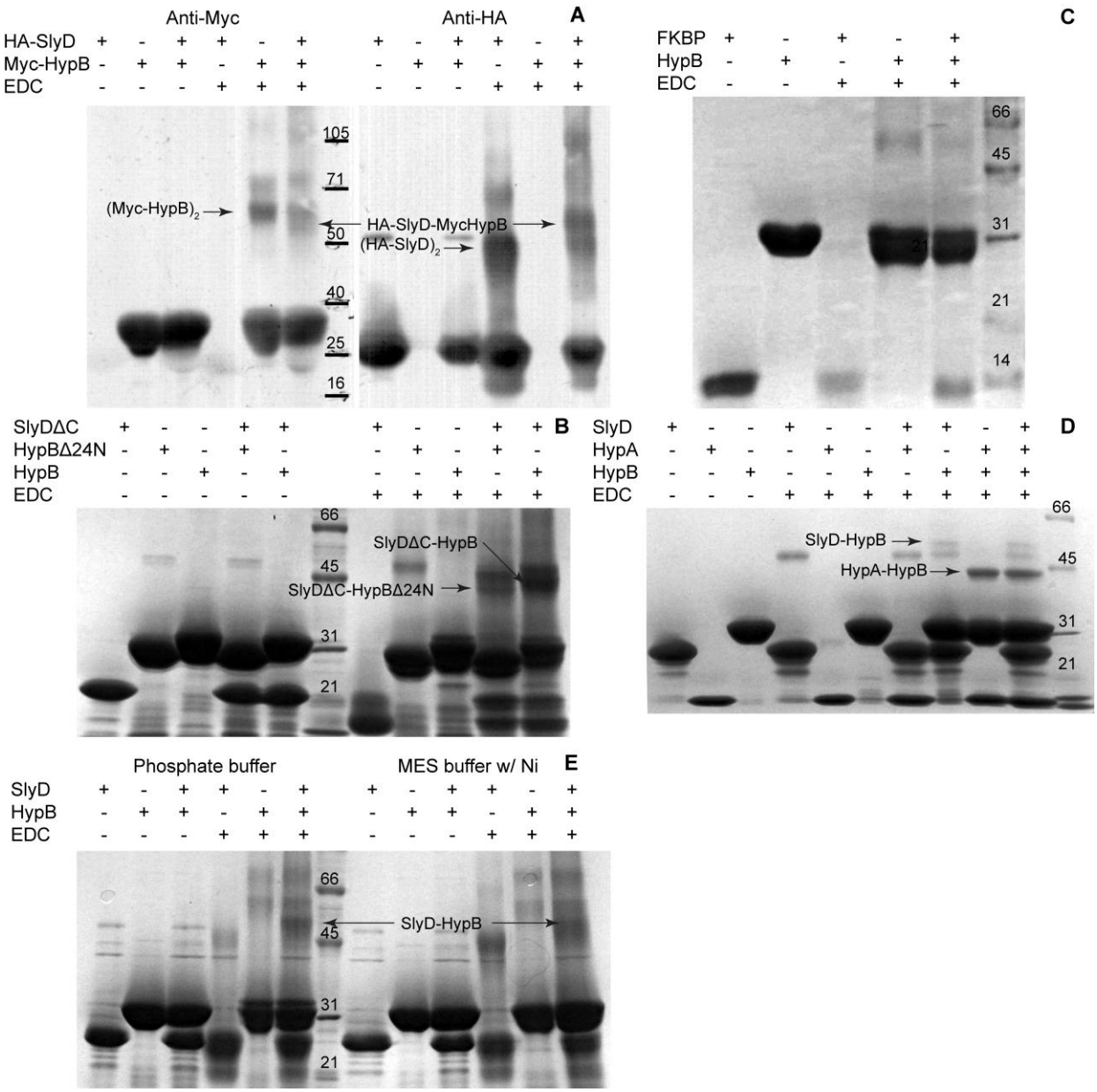

Fig. S1 Cross-linking assays. (A): HA-*Hp*SlyD and Myc-*Hp*HypB form a 1:1 heterodimeric complex (5-15% gradient SDS-PAGE) and were visualized by western blot in parallel by anti-HA and anti-Myc antibodies, respectively. HouBio Rainbow board range (16-220 kDa) Prestained Protein marker. (B): *Hp*SlyDΔC with *Hp*HypBΔ24N/*Hp*HypB (5-15% gradient SDS-PAGE). (C): *Hp*SlyD-FKBP with *Hp*HypB (12% SDS-PAGE). (D): *Hp*SlyD, *Hp*HypA and *Hp*HypB (5-15% gradient SDS-PAGE). Bio-Rad SDS-PAGE Molecular Weight Standards (Broad Range) was used as indicated. (E): *Hp*SlyD with *Hp*HypB in the absence of Ni^{2+} (phosphate buffer as others) and the presence of 150 μM Ni^{2+} (10 mM MES buffer pH 6.5) (5-15% gradient SDS-PAGE). All proteins are in 30 μM. Cross-linking experiments were performed in 10 mM sodium phosphate buffer (pH 6.5), except in (E). Formation of complexes is indicated by arrows.

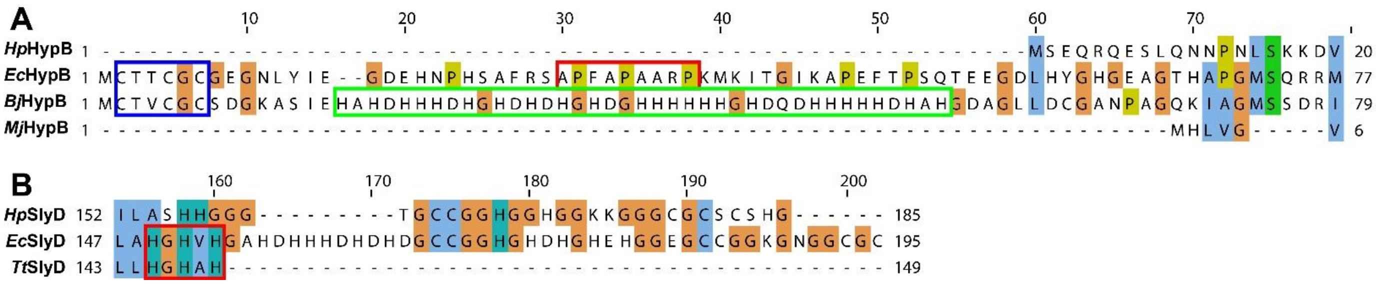

Fig. S2 Protein sequence alignments for HypBs (A) and SlyDs (B). *Bj*: *Bradyrhizobium japonicum*; *Ec*: *Escherichia coli*; *Hp*: *Helicobacter pylori*; *Mj*: *Methanocaldococcus jannaschii*; Tt: *Thermus thermophiles*. Sequences were aligned by using MUSSEL[6] and the alignments were visualized by Jalview.[7] For HypBs, the N-terminal high affinity Ni(II) binding motif CXXCGC is highlighted in blue box, the proline-containing sequence in red box, and N-terminal histidine-rich sequence in green box. For SlyD, the conserved high affinity metal-binding motif HGHXH is highlighted in red box.

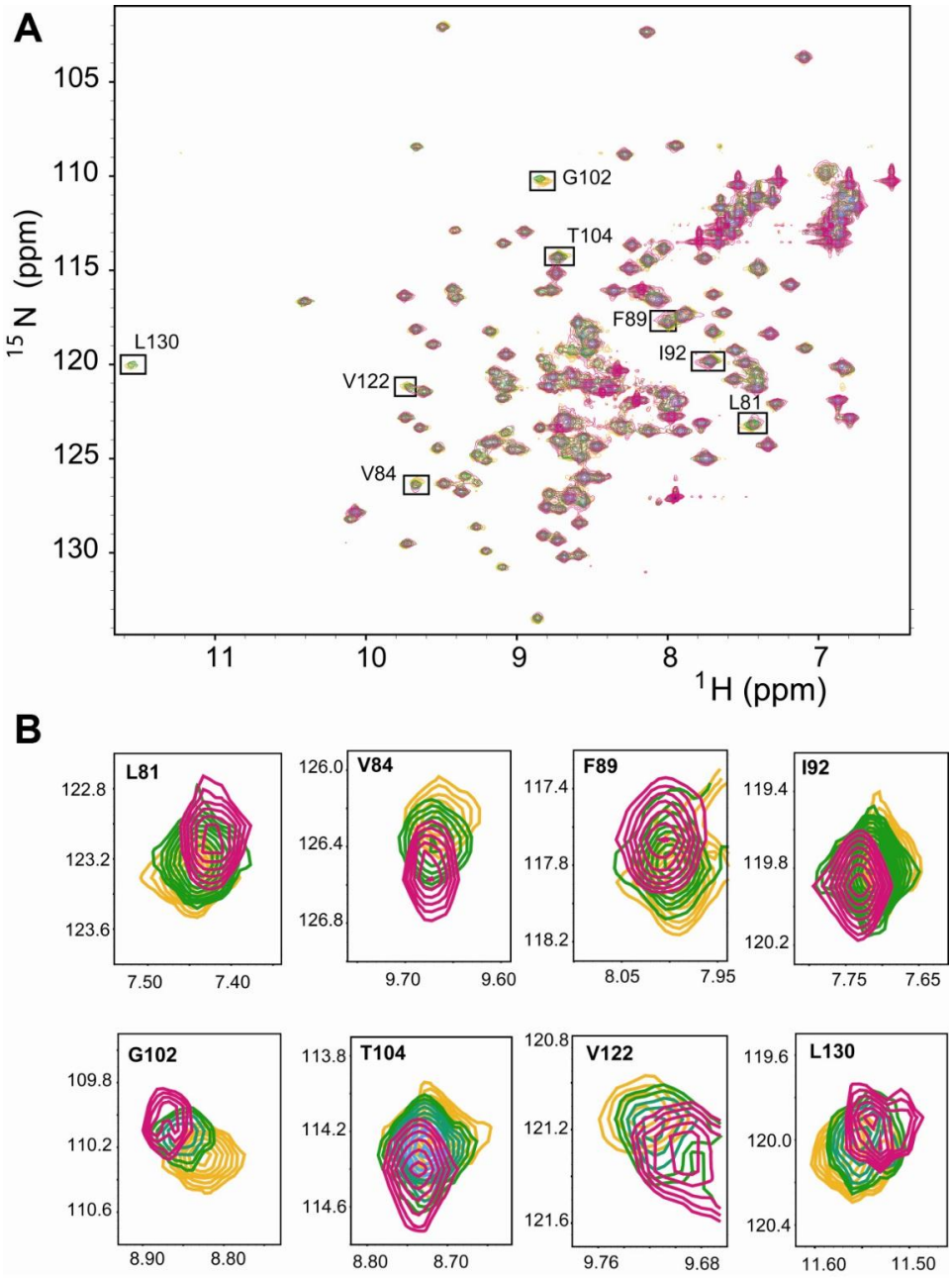

Fig. S3 NMR mapping of *Hp*SlyDΔC upon titration of *Hp*HypB. (A): Overlapped 2D [^{1}H-^{15}N] HSQC spectra of 0.3 mM ^{15}N-labeled *Hp*SlyDΔC titrated with 0 (*yellow*), 0.5 (*green*) and 1 (*magenta*) molar equivalents of *Hp*HypB in 20 mM BisTris-HCl buffer, pH 6.8, containing 100 mM NaCl, 10% (v/v) D_2O, 0.02% NaN_3. (B): Selected cross peaks for residues demonstrating significant chemical shift perturbations.

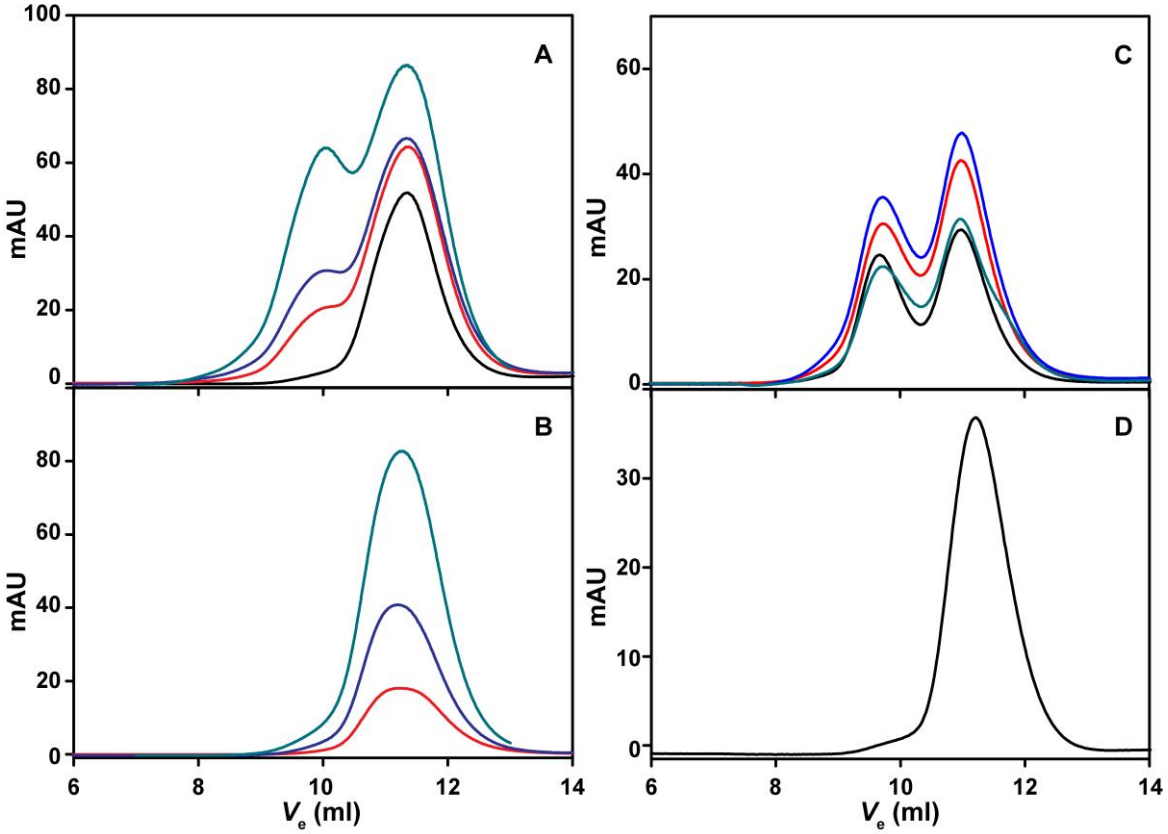

Fig. S4 Size-exclusion chromatography revealed the Ni^{2+} transfer between *Hp*SlyD and *Hp*HypB. (A): Apo-*Hp*HypB (100 µM, ▬) was added with series of concentrations of Ni-bound *Hp*SlyD: 30 µM (▬), 60 µM (▬) and 120 µM (▬); (B): 30 µM (▬), 60 µM (▬) and 120 µM (▬) of Ni-bound *Hp*SlyD; (C) Ni-bound *Hp*HypB (100 µM, ▬) was added with series of concentrations of apo-*Hp*SlyD: 30 µM (▬), 60 µM (▬) and 120 µM (▬) and the profiles are subtracted by corresponding *Hp*SlyD alone; (D) Apo-*Hp*HypB with apo-*Hp*SlyD, 40 µM each.

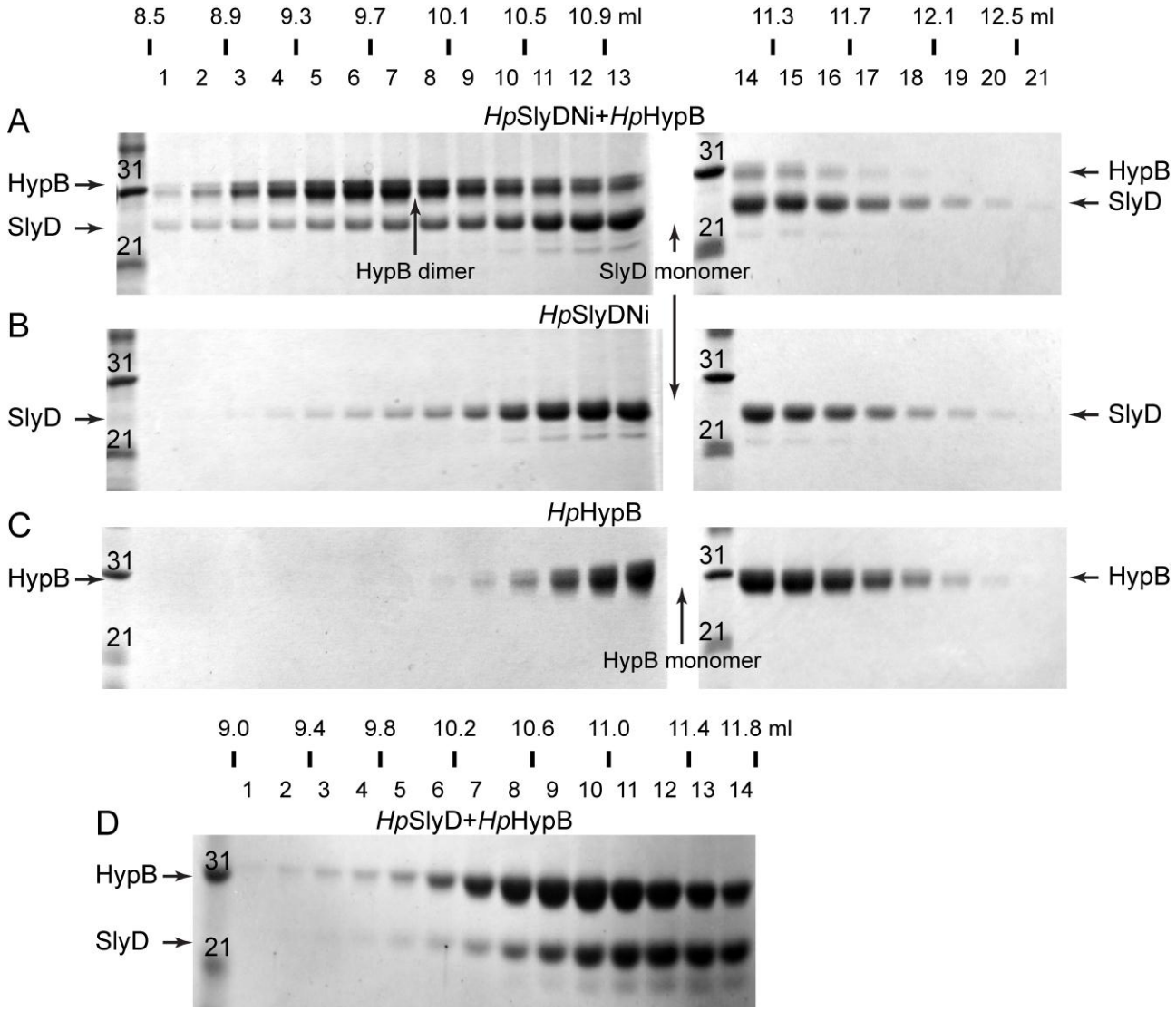

Fig. S5 SDS-PAGE of collected fractions (200 µl each) from elution volume of 8.5 ml for (A) Ni^{2+}-*Hp*SlyD + *Hp*HypB, (B) Ni^{2+}-*Hp*SlyD and (C) *Hp*HypB and from elution volume of 9.0 ml for (D) *Hp*SlyD + *Hp*HypB. The concentration of each protein is 100 µM. The peaks for dimeric *Hp*HypB are centered at around 10 ml.

Table S1 Kinetic parameters of GTPase of *Hp*HypB

	k_{cat} (10^{-4}·s^{-1})	K_m (10^{-5} M)	k_{cat}/K_m (M^{-1}·s^{-1})
HypB	9±1	6±2	15±4
HypBNi	17±1	6±1	29±5
HypBZn	6±1	9±4	7±2
HypB+SlyD	11±1	6±1	19±3
HypB+SlyDNi	16±2	7±2	23±6
HypBNi+SlyD	14±1	3±1	44±9
HypBNi+SlyDNi	16±1	4±1	43±9
HypB+SlyDZn	7±1	3±1	23±9
HypBZn+SlyD	5±1	1±1	35±20
HypBZn+SlyDZn	4±1	4±3	9±4

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