Electronic Supplementary Material (ESI) for Metallomics.

Supporting information

Integration of Fluorescence Imaging with Proteomics Enables Visualization and Identification of Metallo-proteomes in Living Cells

Yau-Tsz Lai[†a], Ya Yang[†a], Ligang Hu[a], Tianfan Cheng[a], Yuen-Yan Chang[a], Mohamad Koohi-Moghadam[a], Yuchuan Wang[b], Jiang Xia[c], Junwen Wang[d,e], Hongyan Li[a], Hongzhe Sun[*a]

---

[a] Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China. E-mail: hsun@hku.hk; Fax: +852 28571586

[b] MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, P.R. China

[c] Department of Chemistry, The Chinese University of Hong Kong, Hong Kong, P.R. China

[d] Center for Individualized Medicine & Department of Health Sciences Research, Mayo Clinic, Scottsdale, AZ 85259 USA

[e] Department of Biological Informatics, Arizona State University, Scottsdale, AZ 85259 USA

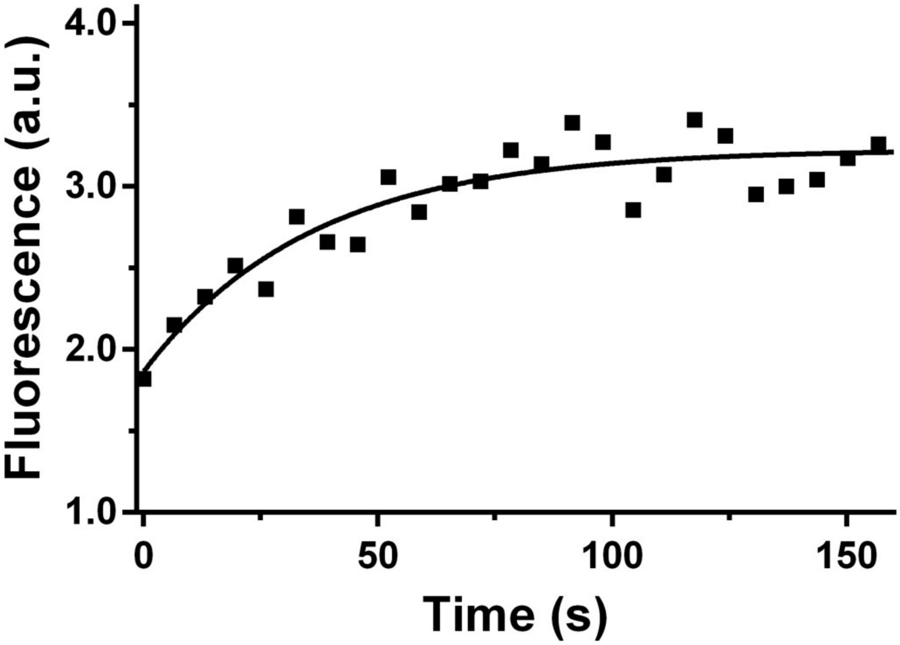

Figure S1. Plot of fluorescence against time upon the displacement of Cu^{2+} from Cu^{2+}-*TRACER* (5 µM) by NTA (500 µM) in 20 mM Tris-HCl buffer, pH 7.2 to determine the dissociation kinetics to be (K_{off}) of 0.018 ± 0.002 s^{-1} by fitting the data with a single exponential rate equation.

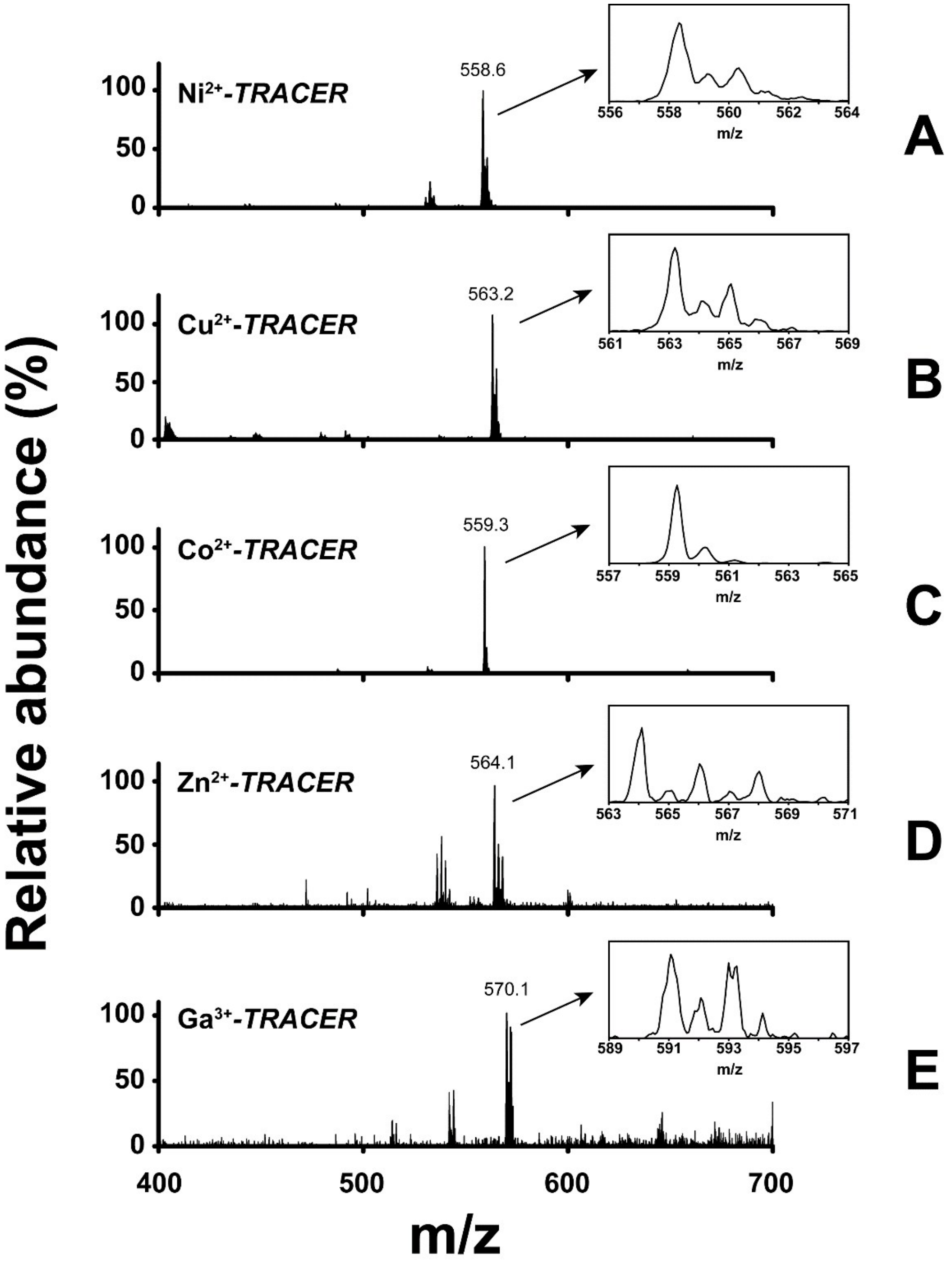

Figure S2. ESI-MS spectra showing 1:1 complexation of *TRACER* with metal ions. **A**) Ni^{2+}-*TRACER*: the peak at 558.6 is assigned to $[M\text{-}3H]^-$ (calcd. 558.9); **B**) Cu^{2+}-*TRACER*: the peak at 563.2 corresponding to $[M\text{-}3H]^-$ (calcd. 563.5); **C**) Co^{2+}-*TRACER*: the peak at 559.3 corresponding to $[M\text{-}3H]^-$ (calcd. 559.4); **D**) Zn^{2+}-*TRACER*: the peak at 564.1 corresponding to $[M\text{-}3H]^-$ (calcd. 564.5); **E**) Ga^{3+}-*TRACER*: the peak at 570.1 corresponding to $[M\text{-}2H]^+$ (calcd. 570.4). *Insets* are showing the patterns of isotopic distribution.

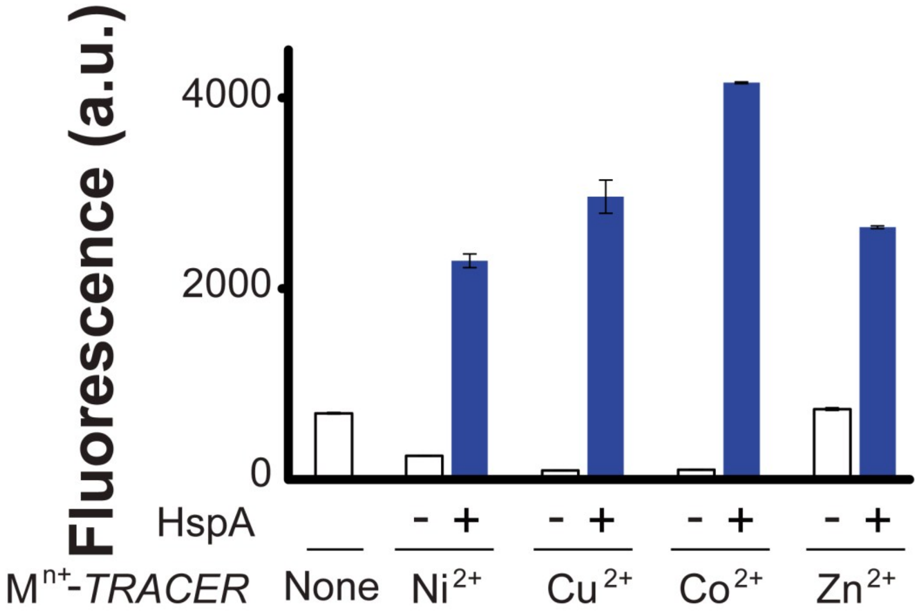

Figure S3. Fluorescence intensities of different M^{n+}-*TRACERs* and their responses upon binding with *Hp*HspA.

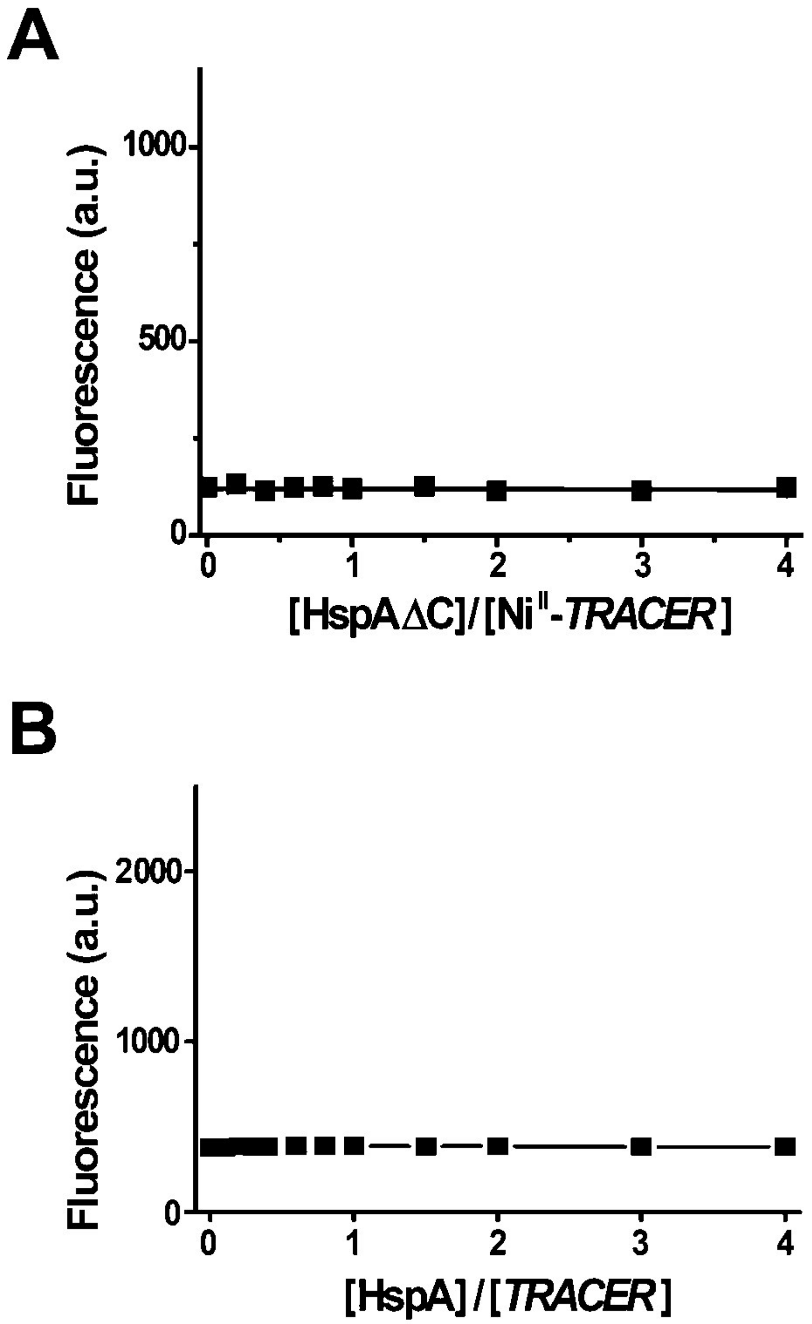

Figure S4. (**A**) Fluorescence response of Ni^{2+}-*TRACER* (5 μM) to different concentrations of *Hp*HspAΔC (0-20 μM). (**B**) Fluorescence response of *TRACER* (5 μM) to different concentrations of *Hp*HspA (0-20 μM). No changes in fluorescence could be observed for Ni^{2+}-*TRACER* reacting with C-terminus-truncated *Hp*HspA (*Hp*HspAΔC) or for *TRACER* alone (without metal ions) upon titration with, indicating that the changes in fluorescence are resulted from the selective binding of chelated metals to the C-terminus of the protein.

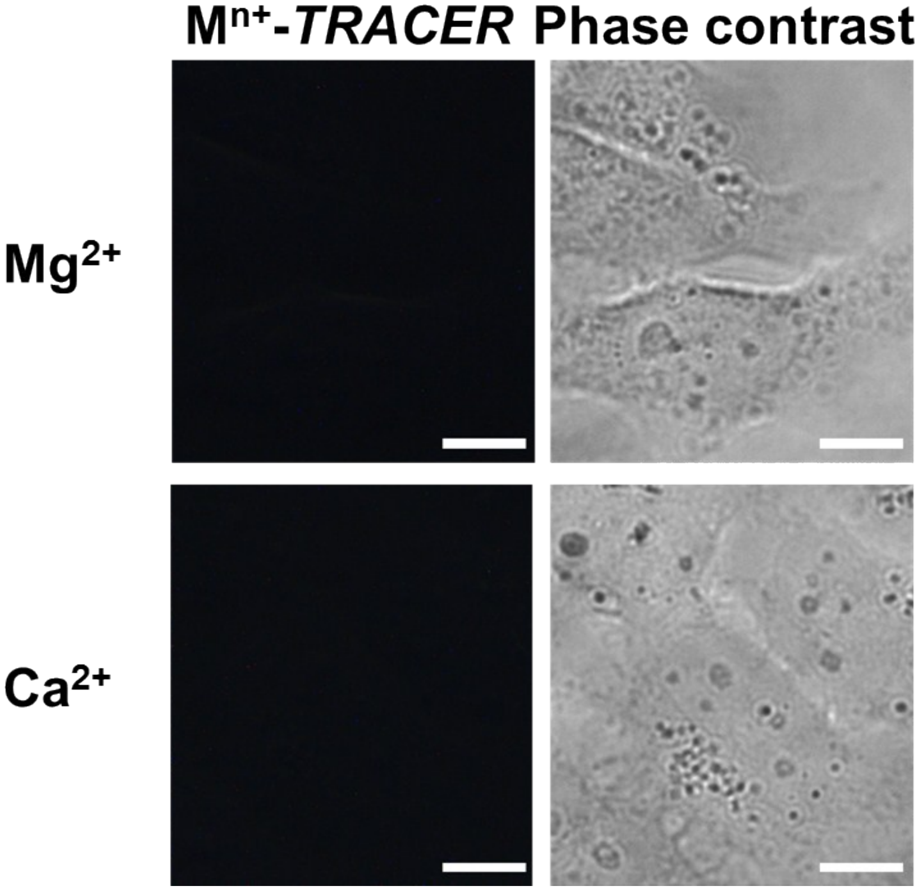

Figure S5. Fluorescent imaging of Hep G2 cells treated with 25 μM of Ca^{2+}-*TRACER* and Mg^{2+}-*TRACER* (n = 3). No fluorescence could be observed in the cells, implying that it is unlikely for either Ca^{2+}-*TRACER* or Mg^{2+}-*TRACER* to enter the cells. Scale bar: 10 μm.

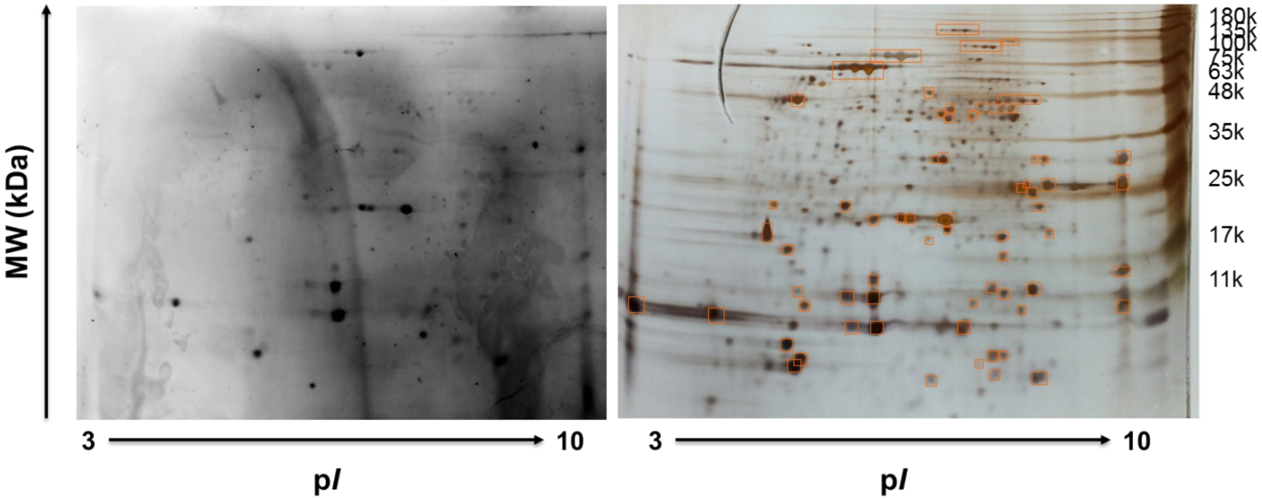

Figure S6. Two-dimensional electrophoresis (2-DE) gel of *H. pylori* 26695 cell lysates pre-incubated with Ni^{2+}-*TRACER* (50 μM). The gel was imaged under UV exposure at 365 nm (*left*) and then silver stained (*right*). The protein spots on the gels selected corresponding to the blue spots were excised and subjected to peptide mass fingerprinting analysis for comparison.

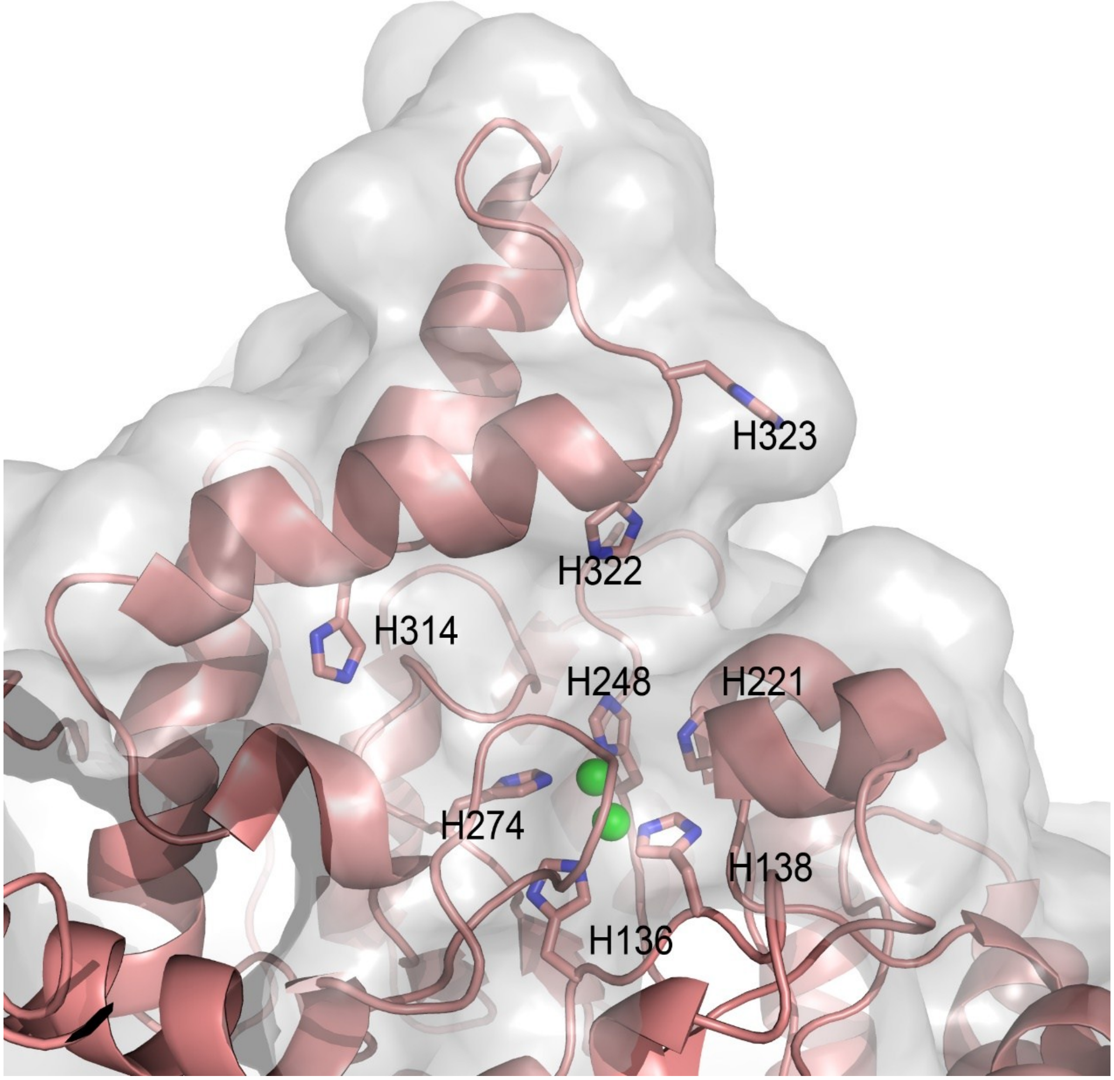

Figure S7. Crystal structure of *H. pylori* UreB (PDBID: 1E9Y) showing spatially histidine-rich region that is proximate to the di-nuclear active center. The protein is shown in cartoon (salmon red) and surface (gray) styles. Histidine residues at or near the active site are shown in stick models, and the di-nuclear nickels are shown green in spheres. The image was generated by PyMOL (Version 1.4.1).

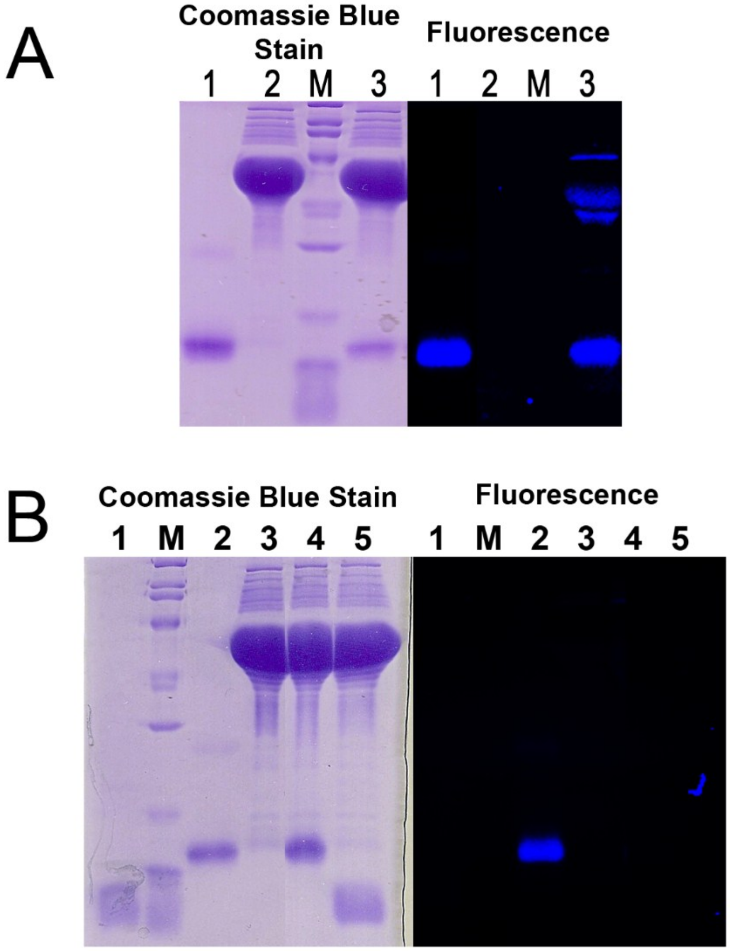

Figure S8. Fluorescence labeling of *Hp*HspA and its partner *Hp*HspB by Ni^{2+}-*TRACER*. **A**) Recombinant *Hp*HspA (*Lane 1*), *Hp*HspB (*Lane 2*) and HspA-HspB complex (*Lane 3*) incubated with Ni^{2+}-*TRACER* (12 μM) were applied to the denaturing gel. *Hp*HspA was lit up while *Hp*HspB alone was not due to lack of histidine-rich motif; however fluorescence was observed on the gel for both proteins upon their complexation. **B**) *Hp*HspAΔC (with histidine-rich C-terminus deleted) (*Lane 1*), *Hp*HspA (*Lane 2*), *Hp*HspB (*Lane 3*), HspA-HspB complex (*Lane 4*) and HspAΔC-HspB complex (*Lane 5*) were incubated with Ni^{2+}-*TRACER* and tris(2-carboxyethyl)phosphine (TCEP)[1] was then added into HspA-HspB complex (*Lane 4*) to allow arylazide of the probe to be reduced. Fluorescent labeling of the protein complexes cannot be achieved when the histidine-rich C-terminus of *Hp*HspA was truncated (*Lane 5*) or the arylazide of Ni^{2+}-*TRACER* was reduced (*Lane 4*). M: protein molecular weight standard (kDa).

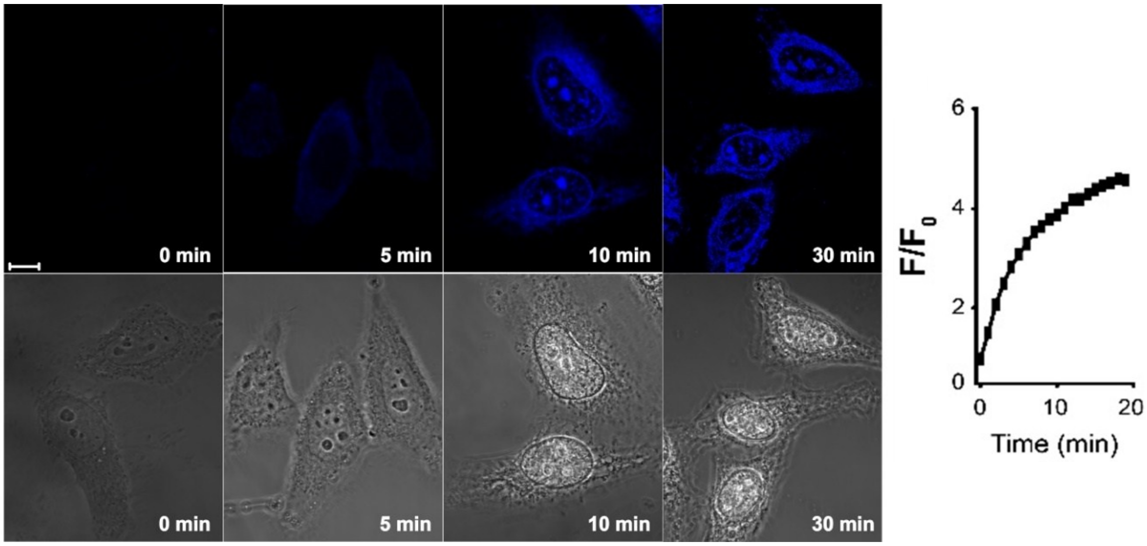

Figure S9. Examination of membrane permeability of Cu^{2+}-*TRACER* upon incubation with HeLa cells. Confocal (*Top*) and phase contrast images (*Bottom*) of HeLa cells obtained every 5 min continuously after supplementation of cells with Cu^{2+}-*TRACER* (25 μM) showing that the probe rapidly enters the cells to label intracellular endogenous metalloproteins. Scale bar: 10 μm

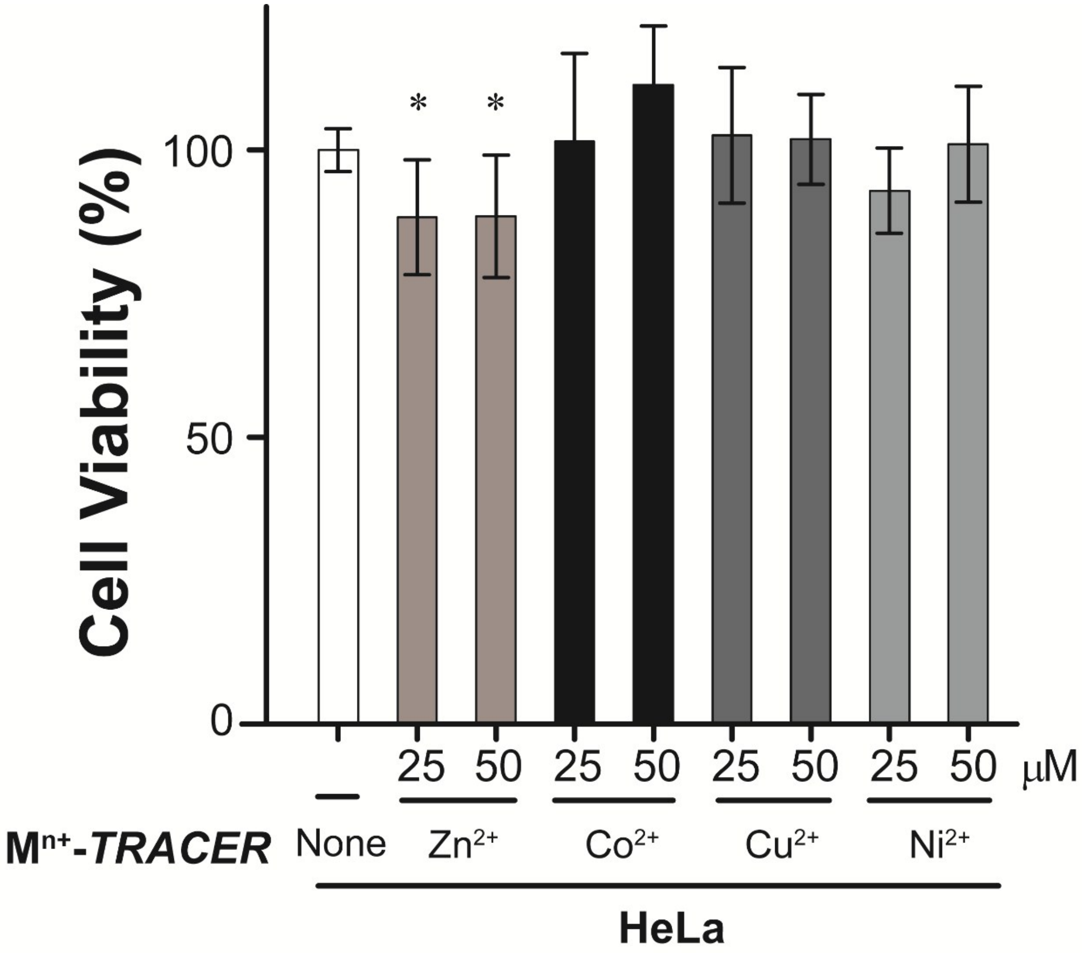

Figure S10. MTT assay on cell viability of HeLa upon treatment of Zn^{2+}-, Co^{2+}-, Cu^{2+}- and Ni^{2+}-*TRACER* with concentrations up to 50 μM. Most probes exhibit negligible cytotoxicity. One-way ANOVA, $^{*}P < 0.05$.

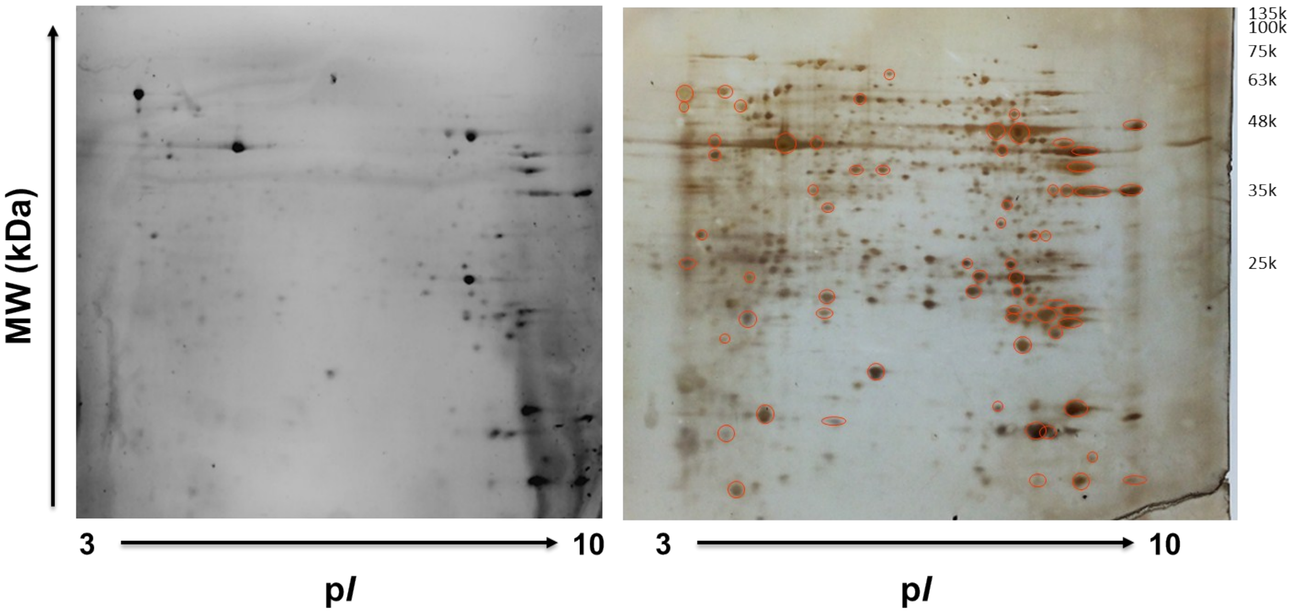

Figure S11. Two-dimensional electrophoresis (2-DE) gel of HeLa cell lysates pre-incubated with Cu^{2+}-*TRACER* (50 μM). The gel was imaged under UV exposure at 365 nm (*left*) and then silver stained (*right*). The protein spots on the gels selected corresponding to the blue spots were excised and subjected to peptide mass fingerprinting analysis for comparison.

Table S1. Proteins identified by Ni^{2+}-*TRACER* in *H. pylori* 26695.

Locus tag	Gene name	Protein name	Entry	Accession No.	IMAC No.
HP0779	acnB	aconitate hydratase B	P56418	gi|487803909	—
HP0192	frdA	fumarate reductase flavoprotein subunit	O06913	gi|446628675	—
HP0695	hyuA	hydantoin utilization protein A	O25402	gi|2313818	—
HP0072	UreB	Urease subunit beta	P69996	gi|15644702	1
HP0010	Hsp60	60 kDa chaperonin	P42383	gi|2506272	2,3
HP0599	HylB	methyl-accepting chemotaxis protein	O25321	gi|446389947	—
HP1205	tufB	translation elongation factor EF-Tu	P56003	gi|2314366	—
HP0197	metK	S-adenosylmethionine synthetase	P56460	gi|570752617	—
HP1507	lys1	saccharopine dehydrogenase	O26037	gi|740634762	—
HP0027	icd	isocitrate dehydrogenase, IDH, IDP	P56063	gi|446246086	—
HP0224	msrAB	peptide methionine sulfoxide reductase MsrA/MsrB	O25011	gi|446789368	5,6
HP1555	tsf	Elongation factor Ts	P55975	gi|15646162	
HP0589	oorA	2-oxoglutarate ferredoxin oxidoreductase subunit alpha	O25311	gi|447129600	
HP0294	amiE	aliphatic amidase	O25067	gi|447138466	
HP0825	trxB	Thioredoxin reductase	P56431	gi|2313959	
HP0176	fba	Fructose-bisphosphate aldolase	P56109	gi|15644805	
HP1111	porB	pyruvate ferredoxin oxidoreductase subunit beta	O25739	gi|446160304	
HP0073	UreA	urease subunit alpha	P14916	gi|446701877	16,17
HP 0175	HP0175	peptidylprolyl isomerase	P56112	gi|446662124	10
HP0410	HpaA	neuraminyllactose-binding hemagglutinin HpaA	O25166	gi|446638365	15
HP0561	fabG	3-ketoacyl-ACP reductase	O25286	gi|447083577	
HP0068	UreG	urease accessory protein	Q09066	gi|15644698	

HP0177	EFP	translation elongation factor EFP	P56004	gi|2313266	
HP1563	tsaA	peroxidase	P21762	gi|570748503	18
HP0389	sodB	superoxide dismutase	P43312	gi|446416778	
HP0620	ppa	inorganic pyrophosphatase	P56153	gi|2313741	19
HP0697	acxC	acetone carboxylase subunit gamma	O25404	gi|487820736	
HP0630	mda66	NADPH quinone reductase MdaB	O25347	gi|446679351	
HP1256	frr	ribosome recycling factor, RRF	P56398	gi|446861076	
HP1118	Ggt	gamma-glutamyltranspeptidase	O25743	gi|447177215	
HP1286	HP1286	hypothetical protein	O25873	gi|446632133	
HP0390	tpx	2-Cys peroxiredoxin	O25151	gi|447097392	
HP0653	Pfr	nonheme iron-containing ferritin (pfr)	P52093	gi|15645277	20,21
HP1038	aroQ	3-dehydroquinate dehydratase	Q48255	gi|446621938	
HP1161	fldA	flavodoxin (fldA)	O25776	gi|15645775	
HP1199	rplL	ribosomal protein L7/L12 (rpl7/l12)	P55834	gi|15645813	
HP0824	trxA	thioredoxin (trxA)	P66928	gi|2313958	
HP0243	napA	DNA protection during starvation protein	P43313	gi|15644871	23
HP0011	GroES	10 kDa chaperonin	P0A0R3	gi|15644644	25
HP1458	HP1458	thiodoxin	O25996	gi|2314636	
HP0902	HP0902	cupin	O25562	gi|446376404	
HP0588	oorD	2-oxoglutarate:acceptor oxidoreductase	O25310	gi|447019638	
HP0105	luxS	S-ribosylhomocysteine lyase	O24931	gi|2313188	
HP1542	HP1542	hypothetical protein HP1542	O26067	gi|2314731	

Table S2. Enriched GO biological process associated with putative Ni^{2+}-binding proteins identified with Ni^{2+}-*TRACER*.

GO ID	GO term	*p*-value	Protein names
GO:0055114	oxidation reduction	1.7E-5	P43313, P52093, O25776, O06913, O25311, O25347, O25310, P56063, O25011, P21762, O25151, P43312, P66928, P56431, O25286
GO:0042592	homeostatic process	3.8E-5	P52093, P21762, P43313, P66928, O25996, O24931, O25151
GO:0019725	cellular homeostasis	5.46E-05	P52093, P21762, P43313, P66928, O25996, O25151
GO:0045454	cell redox homeostasis	0.003759236	P21762, P66928, O25996, O25151
GO:0009405	pathogenesis	0.005927919	Q09066, P14916, P43313, P69996
GO:0006414	translational elongation	0.018218737	P55975, P56004, P56003
GO:0006091	generation of precursor metabolites and energy	0.027058387	O25776, P56418, P66928, O06913, P56109, P56063

*Proteins are listed by Uniprot ID.

Table S3. Enriched GO molecular function associated with putative Ni^{2+}-binding proteins identified with Ni^{2+}-*TRACER*.

GO ID	GO term	*p*-value	Gene names
GO:0046914	transition metal ion binding	0.003669947	P52093, Q09066, P56418, P14916, P43313, P56109, P43312, O24931, O25310, P56460, P69996, P56063
GO:0050662	coenzyme binding	0.003742027	O25776, P56431, O25739, O06913, O25286, O25347, P56063
GO:0016209	antioxidant activity	0.012591503	P56431, P21762, P43312, O25151
GO:0003746	translation elongation factor activity	0.014801126	P55975, P56004, P56003
GO:0046872	metal ion binding	0.016529287	P43313, P14916, P56418, P56109, P43312, O24931, P56460, Q09066, P52093, O25310, P56153, P69996, P56063
GO:0043167	ion binding	0.016529287	P43313, P14916, P56418, P56109, P43312, O24931, P56460, Q09066, P52093, O25310, P56153, P69996, P56063
			P43313, P14916, P56418, P56109,

			P43312, O24931, P56460, Q09066, P52093, O25310, P56153, P69996, P56063
GO:0009055	electron carrier activity	0.022011974	P56431, P66928, O06913, O25310, O25311, O25347
GO:0008135	translation factor activity, nucleic acid binding	0.02567067	P55975, P56004, P56003
GO:0016667	oxidoreductase activity, acting on sulfur group of donors	0.02567067	P56431, O25011, P66928
GO:0005506	iron ion binding	0.038695296	P52093, P56418, P43313, P43312, O24931, O25310
GO:0047553	2-oxoglutarate synthase activity	0.041451416	O25310, O25311
GO:0048037	cofactor binding	0.04622569	O25776, P56431, O25739, O06913, O25286, O25347, P56063

*Proteins are listed by Uniprot ID.

Table S4. Enriched GO cellular component associated with putative Ni-binding proteins identified with Ni^{2+}-*TRACER*.

GO ID	GO term	*p*-value	Protein names
GO:0005737	cytoplasm	2.95E-06	P21762, P14916, P43313, P56460, P56398, P55834, P52093, Q09066, P56431, P55975, P42383, P56004, P56153, P69996, P56003, P0A0R3
GO:0044424	intracellular part	1.90E-05	O25166, P21762, P14916, P43313, P56460, P56398, P55834, P52093, Q09066, P56431, P55975, P42383, P56004, P56153, P69996, P56003, P0A0R3
GO:0005622	intracellular	1.37E-04	O25166, P21762, P14916, P43313, P56460, P56398, P55834, P52093, Q09066, P56431, P55975, P42383, P56004, P56153, P69996, P56003, P0A0R3

*Proteins are listed by Uniprot ID.

Table S5. Proteins identified by Cu^{2+}-*TRACER* in HeLa.

Gene name	Protein name	Accession No.
CALR	calreticulin precursor	gi|4757900
TUBB	tubulin, beta	gi|18088719
P4HB	protein disulfide-isomerase precursor	gi|20070125
CALU	calumenin	gi|2809324
ACTB	beta actin variant	gi|62897671
RCN1	reticulocalbin-1 precursor	gi|4506455
ACTB	actin, beta, partial	gi|14250401
ACTG1	unnamed protein product	gi|194388064
PDIA3	protein disulfide isomerase family A, member 3	gi|119597641
FSCN1	fascin	gi|4507115
ENO1	alpha-enolase isoform 1 (alpha-enolase)	gi|4503571
IDH1	isocitrate dehydrogenase [NADP] cytoplasmic	gi|28178825
ASS1	argininosuccinate synthase	gi|16950633
EEF1A1	elongation factor 1-alpha 1	gi|4503471
PGK1	phosphoglycerate kinase 1	gi|4505763
ALDOA	fructose-bisphosphate aldolase A	gi|4557305
EEF2	elongation factor 2	gi|4503483
EEF2	unnamed protein product	gi|194391174
CAPZA1	F-actin-capping protein subunit alpha-1	gi|5453597
EEF1B2	elongation factor 1-beta	gi|4503477
PGAM1	unnamed protein product	gi|194383738
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	gi|31645
GAPDH	glyceraldehyde-3-phosphate dehydrogenase isoform 1	gi|7669492
TCEANC2	transcription elongation factor A N-terminal and central domain-containing protein 2	gi|23308505
SYT1	synaptotagmin-1 isoform 1	gi|5032139
SMC1A	SMC1A protein, partial	gi|39963673
PGAM1	phosphoglycerate mutase 1 isoform 1	gi|4505753
TPI1	Chain A of triosephosphate isomerase with a single mutant (N71D)	gi|753534216

TPI1	triosephosphate isomerase isoform 1	gi|4507645
HNRNPH1	heterogeneous nuclear ribonucleoprotein H1	gi|119574194
HNRNPH1	heterogeneous nuclear ribonucleoprotein H	gi|5031753
HNRNPH2	heterogeneous nuclear ribonucleoprotein H2	gi|9624998
ARHGDIA	rho GDP-dissociation inhibitor 1 isoform a	gi|4757768
GLO1	lactoylglutathione lyase	gi|118402586
GSTP1	glutathione S-transferase P	gi|4504183
PRDX2	peroxiredoxin-2	gi|32189392
NME1	nucleoside diphosphate kinase A isoform a	gi|38045913
EIF5A	eukaryotic translation initiation factor 5A-1 isoform B	gi|4503545
TUBB	tubulin beta chain isoform c	gi|645912972
TXN	thioredoxin isoform 1	gi|50592994
TAGLN2	transgelin 2	gi|12803567
RAN	GTP-binding nuclear protein Ran isoform 1	gi|5453555
PRDX1	peroxiredoxin-1	gi|4505591
HNRNPA1	HNRPA1 protein	gi|75517570
PEBP1	neuropolypeptide h3, partial	gi|4261934
PEBP1	phosphatidylethanolamine-binding protein 1	gi|4505621
HNRNPA2B1	heterogeneous nuclear ribonucleoproteins A2/B1 isoform A2	gi|4504447
LMNA	lamin A protein, partial	gi|386856
CFL1	cofilin-1	gi|5031635
PPIA	Chain A, human cyclophilin A, Peptidyl-prolyl Cis-trans Isomerase A	gi|380764102
PPIA	PPIA	gi|48145531
PFN1	profilin-1	gi|4826898
SSBP1	Chain A, human mitochondrial single-stranded DNA binding protein	gi|2624694
PFN1	Chain A, human platelet profilin complexed with an L-Pro10-iodotyrosine peptide	gi|5542165

*Proteins highlighted in blue are proteins also identified previously with Cu^{2+}-IMAC.[2]

Table S6. Enriched GO biological process associated with putative Cu^{2+}-binding proteins identified with Cu^{2+}-*TRACER*.

GO ID	GO term	*p*-value	Gene names
GO:0006096	glycolysis	2.44E-07	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0006007	Glucose catabolic process	7.13E-07	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0019320	hexose catabolic process	1.71E-06	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0046365	monosaccharide catabolic process	1.97E-06	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0046164	alcohol catabolic process	3.79E-06	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0007010	cytoskeleton organization	3.88E-06	ACTG1, ALDOA, PFN1, TUBB, RAN, CFL1, FSCN1, CAPZA1, SMC1A, CALR
GO:0044275	cellular carbohydrate catabolic process	4.81E-06	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0016052	carbohydrate catabolic process	1.63E-05	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0006091	generation of precursor metabolites and energy	3.21E-05	ALDOA, TPI1, TXN, PGAM1, IDH1, PGK1, GAPDH, ENO1
GO:0045454	cell redox homeostasis	3.44E-05	P4HB, PDIA3, TXN, PRDX2, PRDX1
GO:0030036	actin cytoskeleton organization	4.86E-05	ACTG1, ALDOA, PFN1, CFL1, FSCN1, CAPZA1, CALR
GO:0030029	actin filament-based process	6.94E-05	ACTG1, ALDOA, PFN1, CFL1, FSCN1, CAPZA1, CALR
GO:0006006	glucose metabolic process	8.29E-05	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0042981	regulation of apoptosis	8.61E-05	TUBB, PDIA3, NME1, CFL1, EIF5A, PRDX2, GLO1, CALR, PRDX1, ARHGDIA, GSTP1
GO:0043067	regulation of programmed cell death	9.36E-05	TUBB, PDIA3, NME1, CFL1, EIF5A, PRDX2, GLO1, CALR, PRDX1, ARHGDIA, GSTP1
GO:0010941	regulation of cell death	9.65E-05	TUBB, PDIA3, NME1, CFL1, EIF5A, PRDX2, GLO1, CALR, PRDX1, ARHGDIA, GSTP1
GO:0019318	hexose metabolic process	2.40E-04	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1

GO:0010035	response to inorganic substance	3.25E-04	ACTG1, ACTB, SYT1, PEBP1, PRDX2, PRDX1
GO:0005996	monosaccharide metabolic process	4.69E-04	ALDOA, TPI1, PGAM1, PGK1, GAPDH, ENO1
GO:0051592	response to calcium ion	5.65E-04	ACTG1, ACTB, SYT1, PEBP1
GO:0051168	nuclear export	7.29E-04	RAN, EIF5A, CALR, HNRNPA1
GO:0000398	nuclear mRNA splicing, via spliceosome	0.00104742	HNRNPH2, HNRNPA2B1, HNRNPH1, SMC1A, HNRNPA1
GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	0.00104742	HNRNPH2, HNRNPA2B1, HNRNPH1, SMC1A, HNRNPA1
GO:0000375	RNA splicing, via transesterification reactions	0.00104742	HNRNPH2, HNRNPA2B1, HNRNPH1, SMC1A, HNRNPA1
GO:0006913	nucleocytoplasmic transport	0.00112569	PDIA3, RAN, EIF5A, CALR, HNRNPA1
GO:0051169	nuclear transport	0.00118011	PDIA3, RAN, EIF5A, CALR, HNRNPA1
GO:0006611	protein export from nucleus	0.00126582	RAN, EIF5A, CALR
GO:0051130	positive regulation of cellular component organization	0.00194516	CFL1, PEBP1, EIF5A, CALR, ARHGDIA
GO:0019725	cellular homeostasis	0.00229589	ALDOA, P4HB, PDIA3, TXN, PRDX2, CALR, PRDX1
GO:0006928	cell motion	0.00252686	ACTG1, ACTB, TUBB, TXN, CFL1, CAPZA1, ARHGDIA
GO:0050658	RNA transport	0.00291332	RAN, HNRNPA2B1, EIF5A, HNRNPA1
GO:0050657	nucleic acid transport	0.00291332	RAN, HNRNPA2B1, EIF5A, HNRNPA1
GO:0051236	establishment of RNA localization	0.00291332	RAN, HNRNPA2B1, EIF5A, HNRNPA1
GO:0006081	cellular aldehyde metabolic process	0.00306945	TPI1, IDH1, GLO1
GO:0006916	anti-apoptosis	0.00310798	CFL1, PRDX2, GLO1, ARHGDIA, GSTP1
GO:0006403	RNA localization	0.00317561	RAN, HNRNPA2B1, EIF5A, HNRNPA1

GO:0006414	translational elongation	0.00326617	EEF1A1, HNRNPH2, EEF1B2, EEF2
GO:0043066	negative regulation of apoptosis	0.00369832	NME1, CFL1, PRDX2, GLO1, ARHGDIA, GSTP1
GO:0043069	negative regulation of programmed cell death	0.00392603	NME1, CFL1, PRDX2, GLO1, ARHGDIA, GSTP1
GO:0060548	negative regulation of cell death	0.00397276	NME1, CFL1, PRDX2, GLO1, ARHGDIA, GSTP1
GO:0015931	nucleobase, nucleoside, nucleotide and nucleic acid transport	0.00447806	RAN, HNRNPA2B1, EIF5A, HNRNPA1
GO:0010038	response to metal ion	0.00647057	ACTG1, ACTB, SYT1, PEBP1
GO:0006405	RNA export from nucleus	0.00649920	RAN, EIF5A, HNRNPA1
GO:0007051	spindle organization	0.00778840	TUBB, RAN, SMC1A
GO:0008380	RNA splicing	0.00959812	HNRNPH2, HNRNPA2B1, HNRNPH1, SMC1A, HNRNPA1
GO:0043603	cellular amide metabolic process	0.01187017	TPI1, ASS1, IDH1
GO:0006979	response to oxidative stress	0.01244645	PEBP1, IDH1, PRDX2, PRDX1
GO:0006575	cellular amino acid derivative metabolic process	0.01285807	P4HB, EIF5A, IDH1, GLO1
GO:0006397	mRNA processing	0.01452517	HNRNPH2, HNRNPA2B1, HNRNPH1, SMC1A, HNRNPA1
GO:0006412	translation	0.01608846	EEF1A1, HNRNPH2, EEF1B2, EIF5A, EEF2
GO:0007015	actin filament organization	0.01912570	ALDOA, CFL1, FSCN1
GO:0043243	positive regulation of protein complex disassembly	0.02051962	CFL1, EIF5A
GO:0042592	homeostatic process	0.02193195	ALDOA, P4HB, PDIA3, TXN, PRDX2, CALR, PRDX1
GO:0016071	mRNA metabolic process	0.02318573	HNRNPH2, HNRNPA2B1, HNRNPH1, SMC1A, HNRNPA1
GO:0042267	natural killer cell mediated cytotoxicity	0.02341728	TUBB, PRDX1
GO:0002228	natural killer cell mediated immunity	0.02341728	TUBB, PRDX1

GO:0006605	protein targeting	0.02540843	PDIA3, RAN, EIF5A, CALR
GO:0045596	negative regulation of cell differentiation	0.02571442	NME1, PRDX2, CALR, ARHGDIA
GO:0051170	nuclear import	0.02780920	PDIA3, RAN, HNRNPA1
GO:0001909	leukocyte mediated cytotoxicity	0.02918754	TUBB, PRDX1
GO:0045730	respiratory burst	0.03778065	PGAM1, PRDX2
GO:0007052	mitotic spindle organization	0.04346814	RAN, SMC1A
GO:0006739	NADP metabolic process	0.04629958	TPI1, IDH1
GO:0042744	hydrogen peroxide catabolic process	0.04912285	PRDX2, PRDX1

Table S7. Enriched GO molecular function associated with putative Cu-binding proteins identified with Cu^{2+}-*TRACER*.

GO ID	**GO term**	***p*-value**	**Gene names**
GO:0003746	translation elongation factor activity	5.24E-05	EEF1A1, EEF1B2, EIF5A, EEF2
GO:0000166	nucleotide binding	5.77E-04	ACTB, EEF1A1, ASS1, RAN, HNRNPA2B1, EEF2, HNRNPA1, ACTG1, TUBB, HNRNPH2, NME1, PEBP1, IDH1, HNRNPH1, SMC1A, PGK1, GAPDH
GO:0008135	translation factor activity, nucleic acid binding	0.00336552	EEF1A1, EEF1B2, EIF5A, EEF2
GO:0016860	intramolecular oxidoreductase activity	0.006066277	P4HB, TPI1, PDIA3
GO:0008379	thioredoxin peroxidase activity	0.012268382	PRDX2, PRDX1
GO:0003697	single-stranded DNA binding	0.012399141	SSBP1, HNRNPA2B1, HNRNPA1
GO:0003779	actin binding	0.017522274	ALDOA, PFN1, CFL1, FSCN1, CAPZA1

GO:0008092	cytoskeletal protein binding	0.018550479	ALDOA, PFN1, NME1, CFL1, FSCN1, CAPZA1
GO:0005200	structural constituent of cytoskeleton	0.021740122	ACTG1, ACTB, TUBB
GO:0051920	peroxiredoxin activity	0.024389969	PRDX2, PRDX1
GO:0003924	GTPase activity	0.026904398	EEF1A1, TUBB, RAN, EEF2
GO:0005525	GTP binding	0.026923563	EEF1A1, TUBB, NME1, RAN, EEF2
GO:0016864	intramolecular oxidoreductase activity, transposing S-S bonds	0.02739763	P4HB, PDIA3
GO:0003756	protein disulfide isomerase activity	0.02739763	P4HB, PDIA3
GO:0032561	guanyl ribonucleotide binding	0.029302304	EEF1A1, TUBB, NME1, RAN, EEF2
GO:0019001	guanyl nucleotide binding	0.029302304	EEF1A1, TUBB, NME1, RAN, EEF2
GO:0016862	intramolecular oxidoreductase activity, interconverting keto- and enol-groups	0.03039625	P4HB, PDIA3
GO:0032553	ribonucleotide binding	0.047821238	ACTG1, ACTB, EEF1A1, TUBB, ASS1, NME1, RAN, PEBP1, EEF2, PGK1, SMC1A
GO:0032555	purine ribonucleotide binding	0.047821238	ACTG1, ACTB, EEF1A1, TUBB, ASS1, NME1, RAN, PEBP1, EEF2, PGK1, SMC1A

Table S8. Enriched GO cellular component associated with putative Cu-binding proteins identified with Cu^{2+}-*TRACER*.

GO ID	**GO term**	***p*-value**	**Gene names**
GO:0005829	cytosol	4.15E-07	ACTB, EEF1A1, EEF1B2, RAN, PGAM1, EIF5A, CALR, ACTG1, TPI1, TUBB, HNRNPH2, NME1, PPIA, TXN, IDH1, ARHGDIA, ENO1
GO:0042470	melanosome	6.12E-06	P4HB, PDIA3, RAN, PEBP1, PRDX1, CALU

GO:0048770	pigment granule	6.12E-06	P4HB, PDIA3, RAN, PEBP1, PRDX1, CALU
GO:0030530	heterogeneous nuclear ribonucleoprotein complex	1.60E-05	HNRNPH2, HNRNPA2B1, HNRNPH1, HNRNPA1
GO:0005788	endoplasmic reticulum lumen	8.93E-05	P4HB, PDIA3, CALR, RCN1, CALU
GO:0005856	cytoskeleton	1.04E-04	ACTG1, ACTB, ALDOA, PFN1, TUBB, HNRNPH2, NME1, CFL1, CAPZA1, FSCN1, LMNA, PEBP1, HNRNPH1, ARHGDIA
GO:0043228	non-membrane-bounded organelle	1.63E-04	ALDOA, ACTB, SSBP1, RAN, FSCN1, HNRNPA2B1, CAPZA1, LMNA, HNRNPA1, ACTG1, PFN1, TUBB, HNRNPH2, NME1, CFL1, PEBP1, HNRNPH1, SMC1A, ARHGDIA
GO:0043232	intracellular non-membrane-bounded organelle	1.63E-04	ALDOA, ACTB, SSBP1, RAN, FSCN1, HNRNPA2B1, CAPZA1, LMNA, HNRNPA1, ACTG1, PFN1, TUBB, HNRNPH2, NME1, CFL1, PEBP1, HNRNPH1, SMC1A, ARHGDIA
GO:0005625	soluble fraction	2.93E-04	ACTG1, ACTB, TPI1, NME1, PEBP1, IDH1, GLO1
GO:0015629	actin cytoskeleton	1.13E-03	ACTG1, ALDOA, PFN1, CFL1, FSCN1, CAPZA1
GO:0031988	membrane-bounded vesicle	1.25E-03	ALDOA, SYT1, P4HB, PDIA3, RAN, PEBP1, PRDX1, CALU
GO:0070013	intracellular organelle lumen	0.00127804	ACTB, P4HB, PDIA3, SSBP1, RAN, HNRNPA2B1, LMNA, CALR, HNRNPA1, CALU, HNRNPH2, CFL1, HNRNPH1, RCN1
GO:0043233	organelle lumen	0.00158249	ACTB, P4HB, PDIA3, SSBP1, RAN, HNRNPA2B1, LMNA, CALR, HNRNPA1, CALU, HNRNPH2, CFL1, HNRNPH1, RCN1
GO:0031974	membrane-enclosed lumen	0.00189830	ACTB, P4HB, PDIA3, SSBP1, RAN, HNRNPA2B1, LMNA, CALR, HNRNPA1, CALU, HNRNPH2, CFL1, HNRNPH1, RCN1
GO:0031982	vesicle	0.00320615	ALDOA, SYT1, P4HB, PDIA3, RAN, PEBP1, PRDX1, CALU

GO:0000267	cell fraction	0.00373696	ACTG1, ACTB, P4HB, TPI1, NME1, LMNA, PEBP1, IDH1, GLO1, CALR
GO:0030529	ribonucleoprotein complex	0.00382561	ACTB, HNRNPH2, HNRNPA2B1, EEF2, HNRNPH1, CALR, HNRNPA1
GO:0016023	cytoplasmic membrane-bounded vesicle	0.00527109	SYT1, P4HB, PDIA3, RAN, PEBP1, PRDX1, CALU
GO:0030863	cortical cytoskeleton	0.00926869	ACTG1, ACTB, CFL1
GO:0031410	cytoplasmic vesicle	0.01096516	SYT1, P4HB, PDIA3, RAN, PEBP1, PRDX1, CALU
GO:0005853	eukaryotic translation elongation factor 1 complex	0.01477883	EEF1A1, EEF1B2
GO:0044432	endoplasmic reticulum part	0.019054535	P4HB, PDIA3, CALR, RCN1, CALU
GO:0005635	nuclear envelope	0.02271992	RAN, LMNA, EIF5A, TAGLN2
GO:0044448	cell cortex part	0.02517894	ACTG1, ACTB, CFL1

References:

(1) Chen, Y.; Kamlet, A. S.; Steinman, J. B.; Liu, D. R. *Nat. Chem.* **2011**, *3*, 146.

(2) Smith, S. D.; She, Y. M.; Roberts, E. A.; Sarkar, B. *J. Proteome Res.* **2004**, *3*, 834.