Supporting Information for:

**Discovery of Cisplatin-binding Proteins by Competitive Cysteinome Profiling**

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Supplementary Table

Table S1: List of cisplatin-binding sites identified by rdTOP-ABPP. [See the attached excel file.]
Figure. S1: Optimization of the conditions of cisplatin incubation for rdTOP-ABPP.

MCF-7 cells were incubated with 0, 100 or 300 μM cisplatin for 1-6 h as indicated. Strong competition on the IAYne probe labeling was observed when cells were incubated with 300 μM cisplatin for 4 h.

Figure. S2: Purification of GLRX1 and TXNDC17. (a) GLRX1 (b) TXNDC17. Wild-type and mutants were recombinantly expressed and purified from E. coli. by nickel-affinity chromatography.
Figure. S3: Purification of MetAP1 and its C14S mutant. WT and mutant of MetAP1 was recombinantly expressed and purified from E. coli. by nickel-affinity chromatography.

Figure. S4: Effect of cisplatin on the activity of purified MetAP1. Activity is measured by monitoring the release of Met from the substrate heptapeptide MAHAIHY. Cisplatin up to 800nM does not show inhibition to the activity of purified MetAP1.
Materials and Methods

Cell culture

MCF-7 cells were maintained and cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) with 10% (vol/vol) FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified atmosphere at 37°C with 5% CO₂. The cells were harvested by scraping and the pellets were washed with phosphate buffered saline (PBS). After centrifugation, the cells were collected, frozen and stored at -80°C.

Plasmid construction

GLRX1, TXNDC17, MetAP1 genes were amplified from Hela cDNAs by PCR, and subcloned into the pet-28a(+) expression vector for purification. All site-specific mutants were generated using the PCR mutagenesis method and verified by sequencing. Primer sequences are designed as following:

GLRX1-F: CTCGAGTGCGGCCGCAAGCTTCTGCAGAGCTCCAATCTGC
GLRX1-R: AGAAGGAGATATACCATGGCTCAAGAGTTTGTGAACTG
GLRX1-C23A-F: CCCCGTACTGCAGGAGGGCCCAAG
GLRX1-C23A-R: CCTGCAGTACGGGGAGGTGGGCTTGA
GLRX1-C26A-F: CCAGGAGGGCCCAAGAGATCCTCAGTCAATTGC
GLRX1-C26A-R: GGGCCCTCCTGGAGTACCAGGCGAGG
TXNDC17-F: TAAGAAGGAGATATACCATGGCCCGCTATGAGGAG
TXNDC17-R:
CTCGAGTGCGGCCGCAAGCTTATCTTCAGAGAACAACATTTCACC
TXNDC17-C43A-F: GGGAAAAGCTGGTCCCCCGACTGCGTGC
TXNDC17-C43A-R: GACCAGCTTTTCCCCCGGCAG
TXNDC17-C46A-F: GGTGCCCCGACTCCGTGCAGGCTG
TXNDC17-C46A-R: GAGTCGGGGCACCAGCTTTTCCCC
MetAP1-F: TATACCATGGCGGCCGCTGGA
MetAP1-R: TGCGGCAAATTGAGACATGAAGTGAGGCC
MetAP1-C14S-F: CCAGCAGTGAGGCCAAGCTC
MetAP1-C14S-R: CTCACTGCTGGAGCGTCTG

Construction of MCF-7 MetAP1 KO cell lines
To generate MCF-7 MetAP1 knockout (KO) cell lines, sgRNA sequences were ligated into LentiCRISPRv2 plasmid and then co-transfected with viral packaging plasmids (psPAX2 and pMD2.g) into HEK293T cells. The cells were replaced with fresh medium at 6 h. MCF-7 cells were plated in 6 cm dishes. Two days post transfection, viral supernatant was filtered through 0.45 μm strainer. MCF7 cells were infected by viral supernatant and selected by 1 mg/mL puromycin for about 1 week. By multiple dilution of positive population into 10 cells per mL and manual checking of single cell, the single positive population was sorted. Loss of MetAP1 protein expression was confirmed by immunoblotting. LenitiCRISPRv2 plasmid, psPAX2 and pMD2.g-based plasmid were generously gifted by Prof. Ying Liu at Peking University.

In-gel fluorescence analysis for cells
After MCF-7 cells were treated by cisplatin (Harveybio) for 1-6 h in the concentration of 100 or 300 μM, they were collected, suspended in PBS with 0.1% Triton X-100 (Sigma-Aldrich) and sonicated on ice. The soluble lysates were collected after ultracentrifugation at 20,000 g for 30 mins at 4 °C. Protein concentration was detected through the BCA protein assay Kit (Pierce) on a microplate reader (Bio-Rad) and quantified to 2 mg/mL. The protein in lysates (100 μL per aliquot) were labeled by the IAYne probe (100 μM) for 1 h at 25 °C. The labeled lysates reacted with 110 μM rhodamine-N₃ in the existence of 1 mM Tris(2-carboxyethyl) phosphine (TCEP, Sigma-Aldrich), 100 μM Tris-(benzyltriazolylmethyl) amine (TBTA) (Sigma-Aldrich), and 1 mM CuSO₄ (Sigma-Aldrich) at room temperature with constant shaking on a thermo mixer for 1 h. After reaction, samples were separated by 10% SDS-PAGE. The image
of rhodamine fluorescence was imaged by ChemiDoc MP (Bio-rad) imaging system. The gel were stained by Coomassie staining (CBB) as loading control.

Quantitative profiling of cisplatin binding proteins and sites.

For quantifying cisplatin binding proteins and sites in proteomes from MCF-7 cells, cells per well were incubated by 300 μM cisplatin in 15 cm dishes overnight in serum-free media (DMEM). The cells were collected, washed by PBS for three times and centrifuged at 1400 g for 3 min. The cells were lysed in 1000 μL of PBS with 0.1% Triton X-100 (Sigma-Aldrich). After sonication on ice, the cell lysates were centrifuged for 20000 g, 30 min at 4 °C to remove the insoluble substances. Protein concentration was detected by using the BCA protein assay Kit and quantified to 2 mg/mL for 1 mL. The protein in lysates (1000 μL per aliquot) were labeled by the lAyne probe (100 μM) for 1 h at 25°C. Cell lysates were then reacted with 1 mM CuSO₄, 100 μM TBTA ligand, 110 μM acid-cleavable azide-biotin tag and 1 mM TCEP for 1 h on thermo mixer at 29°C. The labeled proteins of click-labeled lysates were centrifuged for 8000 g, 5 min at 4 °C and washed three times with 1 mL cold methanol.

The precipitated proteomes were resuspended in 1 mL PBS with 1.2% SDS. The samples were sonicated and heated at 90 °C for 10 min to dissolve the proteins, followed by centrifuging at 19000 g, 2 min at room temperature to remove the remaining cupric ion. The solution was diluted with 5 mL of PBS containing 100 μL of prewashed streptavidin Beads (Thermo Fisher Scientific) and incubated for 3 h at 29 °C on a rotator. The beads were washed by 5 mL PBS and 5 ml distilled water and were centrifugated in 1400 g, 3 min for three times. The resulting beads were resuspended in 500 μL PBS with 6 M urea and 10 mM DTT (200 mM DTT as storage) at 37 °C for 30 min and alkylated (400 mM DTT as storage) by addition of 20 mM iodoacetamide at 35 °C for 30 min in the dark. The beads were washed and collected by centrifugation (1700 g, 5 min) and resuspended in 200 μL of 100 mM triethylammonium bicarbonate
(TEAB) buffer (Sigma) with 1 M urea, 1 mM CaCl₂ and 10 ng/μL trypsin (Promega). Trypsin digestion was performed at 37°C in a thermomixer for 16 h. The beads were collected by centrifugation and washed with 1 ml, 100 mM TEAB buffer for three times, and suspended in 100 μL TEAB buffer. The peptides on beads were reacted with 8 μL of 4% D³¹CDO (Sigma) and 8 μL of 0.6 M NaBH₃CN for samples from cisplatin treated, HCHO (Sigma) and 8 μL of 0.6 M NaBH₃CN for samples from normal cells. The reaction was incubated for 2 h at 29°C on a thermo mixer and the beads were washed with 1 mL H₂O for four times. The labeled proteins on beads were combined and cleaved by using 200 μL of 2% formic acid solution (sigma) for twice. The eluted peptides were collected for LC-MS/MS analysis.

**Identification of cisplatin-adducted peptides from purified GLRX1 and TXNDC17 by LC-MS/MS**

For identifying cisplatin-adducted peptides from purified GLRX1 and TXNDC17, proteins were quantified by using the BCA protein assay Kit into 1 mg/mL for 100 μL. 100 μM TCEP was added into proteins for 30 min at room temperature. Each protein was incubated by 300 μM cisplatin for 1 h. Excessive cisplatin was removed and the buffer was changed from PBS into 100 mM TEAB with 6 M urea by filter. The samples were incubated with 10 mM DTT for 30 min at 37°C, and alkylated (400 mM DTT as storage) by addition of 20 mM iodoacetamide at 37°C for 30 min in the dark. The buffer was changed into 100 mM TEAB with 2 M urea by filter. Trypsin digestion was performed at 37°C in a thermomixer for 16 h. Collected peptides flowed through the filter, desalted by C18 tips. The eluted peptides were collected for LC-MS/MS analysis.

**LC-MS/MS and data analysis**

Samples were analyzed by LC-MS/MS on Q Exactive-plus series Orbitrap
mass spectrometers (Thermo Fisher Scientific) coupled with EasyNano-LC. Mobile phase A was 0.1% FA in H$_2$O, and mobile phase B was 0.1% FA in 80% ACN. The flow rate was 3 μL/min for loading and 0.3 μL/min for eluting. Labeled peptide were loaded onto a 100 μm fused silica column packed with 14 cm × 3 μm C18 resin. Peptides were eluted using a 85 min gradient (0 min 5% B; 15 min 5% B; 20 min 10% B; 65 min 40% B; 65.1 min 95% B; 75 min 95% B; 75.2 min 5% B and 85 min 5% B) with a flow rate of 300 nL/min. Under the positive-ion mode, full-scan mass spectra were acquired over the m/z range from 350 to 1800 using the Orbitrap mass analyzer with mass resolution of 70,000. MS/MS fragmentation is performed in a data-dependent mode. The TOP 20 most intense ions are selected for MS2 analysis a resolution of 17,500 using collision mode of HCD. Other important parameters: isolation window, 2.5 m/z units; default charge, 2+; normalized collision energy, 28%; maximum IT, 50 ms; and dynamic exclusion, 18.0 s.

For rdTOP-ABPP, LC-MS/MS data was analyzed by ProLuCID with static modification of cysteine carbamidomethylation (+57.0215 Da). Additional modifications include N-terminus dimethyl labeling and lysine residue dimethyl labeling (+28.03130 Da (light) and + 34.06312 Da (heavy)) and variable IAyne probe modification of cysteine (+223.16846 Da) and oxidation of methionine (+15.9949 Da). The ratios were quantified by the CIMAGE 2.0 software as described previously. For the identification of cisplatin adducted peptides, LC-MS/MS data was analyzed by pLINK. Additional linker modifications include Cisplatin (+227.01 Da), Cisplatin-H$_2$O(+245.02 Da) and Cisplatin-2H$_2$O (+263.03 Da) between the α-site cysteine and the β-site cysteine.

**Purification of target proteins with 6xHis tags from E.coli.**

Full-length complementary DNA of GLRX1, TXNDC17 and MetAP1 were cloned from the cDNA of Hela cells by PCR, and then subcloned into pet28a (+) plasmid containing 6xHis tag on C-terminal sides. All site-specific mutants were generated using the PCR mutagenesis method and verified by
sequencing. All plasmids were transferred into E. coli BL21 (DE3) cells. The
cells were cultured in LB media (containing 5 g yeast extract, 10 g NaCl and 10
g tryptone per 1L) at 37°C until OD at 600 nm reached between 0.6 and 1.5.
The protein overexpression was induced by the addition of IPTG to a final
concentration of 0.5 mM. After IPTG induction at 18°C for 20 hours, bacterial
cells were collected by centrifuging at 4000 rpm for 30 min, and frozen at -80°C
until further use. The GLRX1-overexpressing bacteria were resuspended in
PBS with 1 mM PMSF and 10 mM imidazole. The TXNDC17-overexpressing
bacteria were resuspended in the buffer containing 20 mM Heps, pH 7.2, 500
mM NaCl, 1 mM PMSF. The MetAP1-overexpressing bacteria were
resuspended in the buffer containing 25 mM Tris, pH 8.0, 500 mM NaCl, 1 mM
PMSF. The bacteria cells were lysed by sonication. The supernatant was
collected after centrifugation at 12,000g for 30 mins and loaded on Ni-chelating
column (GE Healthcare, USA). The target proteins were eluted by imidazole
gradient at a concentration of 10 – 500 mM. GLRX1, TXNDC17 and MetAP1
were subjected to a final purification step by size-exclusion chromatography
(Superdex200 Increase 10/300 GL, GE Healthcare) in PBS, pH 7.4; 20 mM
HEPES, 150 mM NaCl pH7.2 and 25 mM Tris, 150 mM NaCl, pH 8.0
respectively.

In-gel fluorescence analysis for purified proteins

The concentration of GLRX1, TXNDC17 and MetAP1 were detected
through the BCA protein assay Kit (Pierce) on a microplate reader (Bio-Rad)
and quantified to 0.1 mg/mL. For GLRX1 and TXNDC17, 100 μM TCEP was
added and incubated for 30 min in advance. All three proteins were incubated
by 300 μM cisplatin for 1 h and labeled by the IAyne probe (100 μM) for 1 h at
25°C. The labeled lysates reacted with 110 μM rhodamine-N₃ in the existence
of 1 mM TCEP, 100 μM TBTA, and 1 mM CuSO₄ at room temperature with
constant shaking on a thermomixer for 1 h. After reaction, samples were
separated by 10% SDS-PAGE. The image of rhodamine fluorescence was imaged by ChemiDoc MP (Bio-rad) imaging system. The gels were stained by Coomassie staining (CBB) as loading control.

Inductively coupled plasma mass spectrometry (ICP-MS)

200 μL nitric acid (MOS level) and 20 μL hydrogen peroxide (MOS level) were added to each protein sample and incubated at 70°C for 1-5h. Then the samples were diluted with ddH₂O into 5 mL and the metal contents were analyzed by ICP-MS using an Elan DRC-e (PerkinElmer) spectrometer. The protein concentrations were determined by Nanodrop.

Measurement of the activity of purified MetAP1

Methionine aminopeptidase activity was evaluated by quantifying the release of Met from the heptapeptide MAHAIHY (GenScript) as adapted from (Weiss et al. 2022). Reactions were conducted at 25°C in 25 mM HEPES pH 7.4, 150 mM NaCl, 2 mM TCEP and consisted of 200 nM human METAP1 with 2 molar equivalents of Zn. Reactions were initiated with the addition of 750 μM of MAHAIHY peptide into 100 μL proteins with or without varying concentration of cisplatin. The reaction was quenched with the addition of 20 μl glacial acetic acid after 120 s. 120 μl of 4% w/v ninhydrin (Sigma-Aldrich) in 100% ACN was added to each quenched reaction and incubated at 37°C for 15 min to detect free Met. The absorbance at 570 nm was measured and the cleaved Met concentration was determined from a standard curve of 10-750 μM Met. All data are reported as the average of triplicate measurements from each evaluated experimental condition.

Measurement of the activity of MetAP1 in living cells

The wild-type MCF-7 cell lines and MetAP1 knockout (KO) cell lines were treated by Bengamide B (0.1 μM, Santa cruz) or cisplatin (5 μM) for 24 h. They
were collected, suspended in PBS with 0.1% Triton X-100 and sonicated on ice. Protein concentration was detected through the BCA protein assay Kit on a microplate reader and quantified to 2 mg/mL. The abundance of MetAP1 (Abcam), β-actin (Abcam), the total 14-3-3γ (Abcam), and the N-terminal initiator methionine unprocessed form of 14-3-3γ (Novus) were shown by immunoblotting (IB) with specific antibodies. The band intensities were quantified by ImageJ and the levels of iMet 14-3-3γ were normalized to the total 14-3-3γ. β-actin was used as loading controls. Bengamide B is a commonly used inhibitor for MetAP1 and MetAP2.