**SUPPLEMENTARY INFORMATION**

## METHODS

### Reagents

All common reagents were obtained from Thermo Fisher Scientific (Waltham, MA) or Sigma-Aldrich (St. Louis, MO). Difluoro-4-methylumbelliferyl phosphate (DiFMUP) was obtained from Invitrogen (cat # D22065). Solid black 384-well plates (cat# 3573) for phosphatase assays were from Corning Incorporated (Corning, NY).

### Antibodies

The antibodies used in the study were purchased from: Upstate Biotechnology (pTyr, catalog 05-321, clone 4G10;); Invitrogen (pY1162/1163-IR-β, catalog 700393, clone 97H9L7); Santa Cruz Biotechnology Inc. (IR-β, catalog sc-711, clone C711); and Cell Signaling Technology (JAK2, catalog 3230, clone D2E12).

### Site directed mutagenesis

Site-directed mutagenesis was performed using QuikChange multisite-directed mutagenesis kit (Stratagene) as per the users manual. PTP1B (1-405) cloned in the pet21b vector was used as template to generate all the mutants.

### Purification of wild-type and mutant forms of PTP1B

Wild-type and mutant forms of PTP1B containing a His-tag were expressed in *Escherichia coli* BL21 (DE3)-RIL using LB. Cells were resuspended in lysis buffer (50 mM Hepes pH 7.2, 150 mM NaCl, 10 mM imidazole, 2 mM TCEP) with cOmplete EDTA-free Protease Inhibitor Cocktail (Roche), and then lysed using a sonicator at 4°C. Lysates were clarified by centrifugation. Initial protein purification was by gravity flow, nickel column chromatography. Proteins were used immediately or stored at -80°C in in 50 mM HEPES pH 7.4, 100 mM NaCl, 2 mM dithiothreitol and 25 % glycerol.

### Gel filtration

PTP1B (5 μM) was incubated with 10 μM MSI-1436 in a final volume of 200 μl buffer (50 mM HEPES, pH 7.0, 100 mM NaCl, 0.2 mM EDTA, 2 mM DTT) at 4 °C. The reaction mixture was then subjected to gel filtration on a Superdex 200 column (HR30/10; Pharmacia Biotech Inc.). Prior to loading the MSI-1436–saturated protein sample, the

column was equilibrated with 50 mM HEPES, pH 7.0, 100 mM NaCl, 0.2 mM EDTA and 2 mM DTT. The void volume ($V_o$) and total volume ($V_t$) were measured using blue dextran and Coomassie blue dye, respectively. Fractions of 0.5 ml were collected, and the protein concentration in each sample was determined by Bradford assay or by recording the UV absorption spectrum at 280 nm.

**Copper-binding assays**

Direct binding assays were performed using radiolabelled copper (64 $Cu^{2+}$). Varying concentration of radiolabeled copper (0-10 uM) was incubated with DPM-1001 (100 nM). Excess copper was removed by running the samples through a desalting column. The amount of metal bound to the compound was quantitated directly by scintillation counting. For assays with the protein, His-tagged PTP1B (100 nM) was incubated with varying concentrations of Cu-DPM-1001 complex for 60 min in assay buffer (50 mM Hepes, 100 mM NaCl, 0.1% BSA pH 6.5) at 25 °C. Protein-bound and free compound were separated by incubating the protein-inhibitor mixture with 50 µl of 50% Ni-NTA beads for 10 min at 25 °C. The beads were washed with assay buffer containing 150 mM NaCl and 25 mM Imidazole three times, and the inhibitor bound to protein was determined by scintillation counting. The amount of bead-bound protein was estimated by BCA protein quantification.

**Phosphatase activity assays**

PTP assays were performed in black polystyrol 96-well plates using DiFMUP as substrate. DiFMUP (10 µM) was added to assay buffer (50 mM Hepes, 100 mM NaCl, 0.1% BSA, 2 mM DTT, 2 mM EDTA, pH 6.5) containing 10 nM purified PTP1B and DPM-1001 (0-10 µM) in a final volume of 100 µl. The fluorescence emitted at 450 nm was monitored continuously for 240 min using a Gemini XPS fluorescence plate reader. For assays using Catalase and Peroxiredoxin, similar assays were performed in the presence of Catalase (25 nM) or Peroxiredoxin (10 nM).

**ESI-MS analysis of DPM-1001-Copper complex**

Eight equivalents of $M(NO_3)_2 \cdot xH_2O$ or $MSO_4 \cdot xH_2O$ (M = Cu, Zn) were reacted with 1 eq. of DPM-1001 (1 mM in $H_2O$). The reaction solutions were stirred at RT, 40 $^oC$ and 80 $^oC$ for 2 h. Mass spectra were recorded on a Bruker Esquire 6000 HCT quadrupole ion trap instrument (Bruker Daltonik GmbH, Leipzig, Germany), equipped with an electrospray

ionization (ESI) source. Generation of the molecular formula of the DPM-1001/Cu(II) species and the comparison between the theoretical (simulated) and the measured isotopic patterns was performed using the DataAnalysis 4.0 software (Bruker).

**Animal experiments**

All protocols were approved by the Institutional Animal Use and Care Committee of Cold Spring Harbor laboratory. 10 week-old male mice fed chow diet or high-fat diet (D12492) were acclimatized for 10 days under standard conditions before experiments. Mice were injected intraperitoneally (i.p.) once daily with vehicle, 5 mg/kg of DPM-1001 intraperitoneally or orally for 50 days. Mice were euthanized after 6 h of fasting, and serum samples were collected to measure the metabolic parameters and tissue samples were collected for studying changes in signaling.

**Metabolic measurements**

Glucose in tail blood was measured using a glucometer (One-Touch Basic; Lifescan, CA). For glucose tolerance tests (GTTs), mice were fasted for 10 hours and then injected 20% D-glucose (2 mg/g body weight) and the blood glucose was monitored immediately before and at 15, 30, 60 and 120 mins following the injection. For insulin tolerance tests (ITTs), 4-h fasted animals were given insulin (0.75 mU/g) and blood glucose was measured immediately before and at 30, 60 and 120 minutes postinjection. Statistical analysis was performed using ANOVA for both GTT and ITT.

**Immunoblotting**

To analyze changes in tyrosine phosphorylation of the insulin receptor (IR), liver samples obtained from saline- or DPM-1001-treated mice were lysed in degassed RIPA lysis buffer (25 mM HEPES pH 7.5, 150 mM NaCl, 0.25% deoxycholate, 10% glycerol, 25 mM NaF, 1 mM EDTA, 1 % Triton, and complete protease inhibitor cocktail). Soluble proteins were harvested by centrifugation at 13,000xg for 10 min at 4 C and quantitated. Equal quantities of lysate, by total protein, were subjected to immunoprecipitation using 4G10-platinum antibody, following which samples were resolved by SDS-PAGE, transferred to nitrocellulose and probed with antibody to the insulin receptor. For total-IR (below pY-IR in Figure 3), a separate gel was loaded with an equal amount of lysate for all samples, resolved by SDS-PAGE, transferred to nitrocellulose and probed with antibody to the insulin receptor. Proteins were detected by enhanced

chemiluminescence (ECL). The same procedure was followed to analyze changes in tyrosine phosphorylation of JAK2 in lysates of hypothalamus. Image J software was used to quantitate blots and the data was plotted using GraphPad Prism, version 7 (GraphPad Software).

**Statistics**

Statistical analysis and tests were chosen based on established protocols and performed using GraphPad Prism, version 7. *P*-values can be found in corresponding Figure Legends.

## SUPPLEMENTARY FIGURE LEGENDS

### Supplementary Figure 1

**A.** ESI-MS spectrum of DPM-1001 reacted with $Cu(NO_3)_2$. Inset: isotopic pattern analysis of the peaks at *m/z* 692.5 and 755.5.

**B.** Proposed structures for the complexes [Cu(DPM-1001)(NO3)] (**I**) and [Cu(DPM-1001)(NO3)2] (**II**).

### Supplementary Figure 2

DPM-1001 (1 mM) was incubated with $ZnSO_4$ or $ZnNO_3$ (8 mM) and the reaction mixture was analyzed by ESI-MS

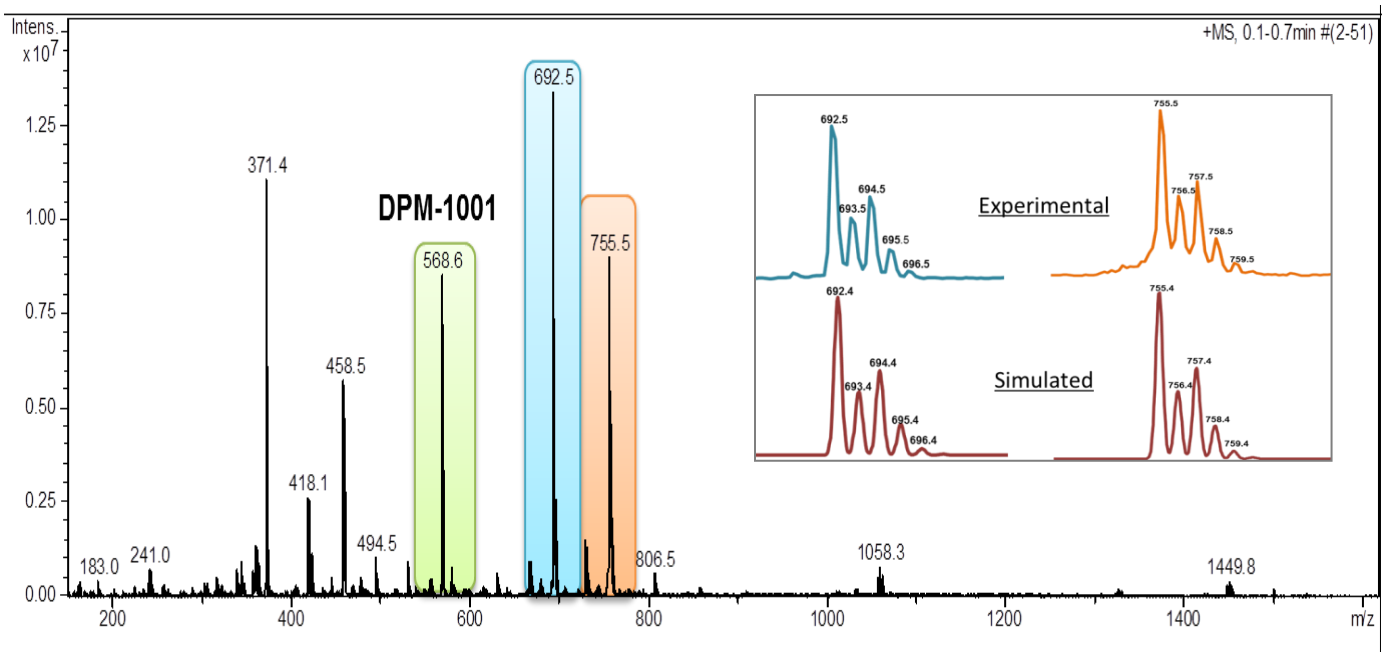
### Supplementary Figure 3

A. The effect DPM-1001 (10 μM) on the activity of a panel of PTPs was tested as indicted.

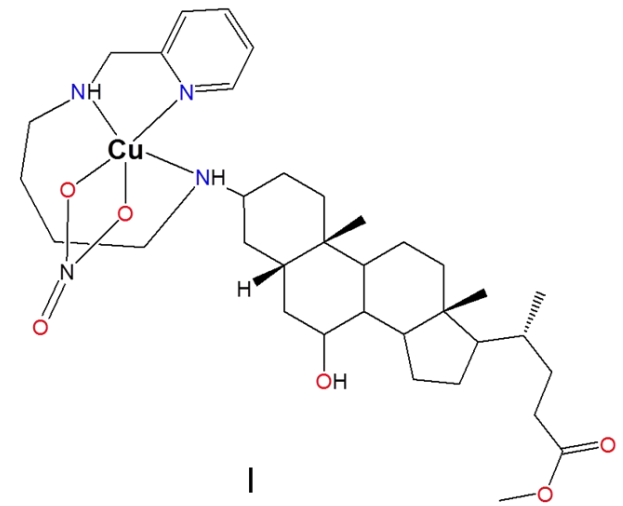
B. Binding of radiolabelled $^{64}Cu$ to the long ($PTP1B_{1-405}$) and short ($PTP1B_{1–321}$) forms of PTP1B revealed saturation at concentrations above 1 mM.

Supplementary Figure 1

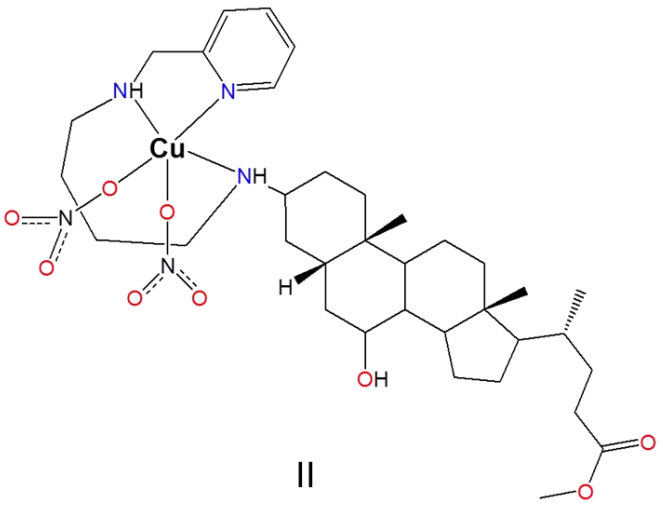
**A**



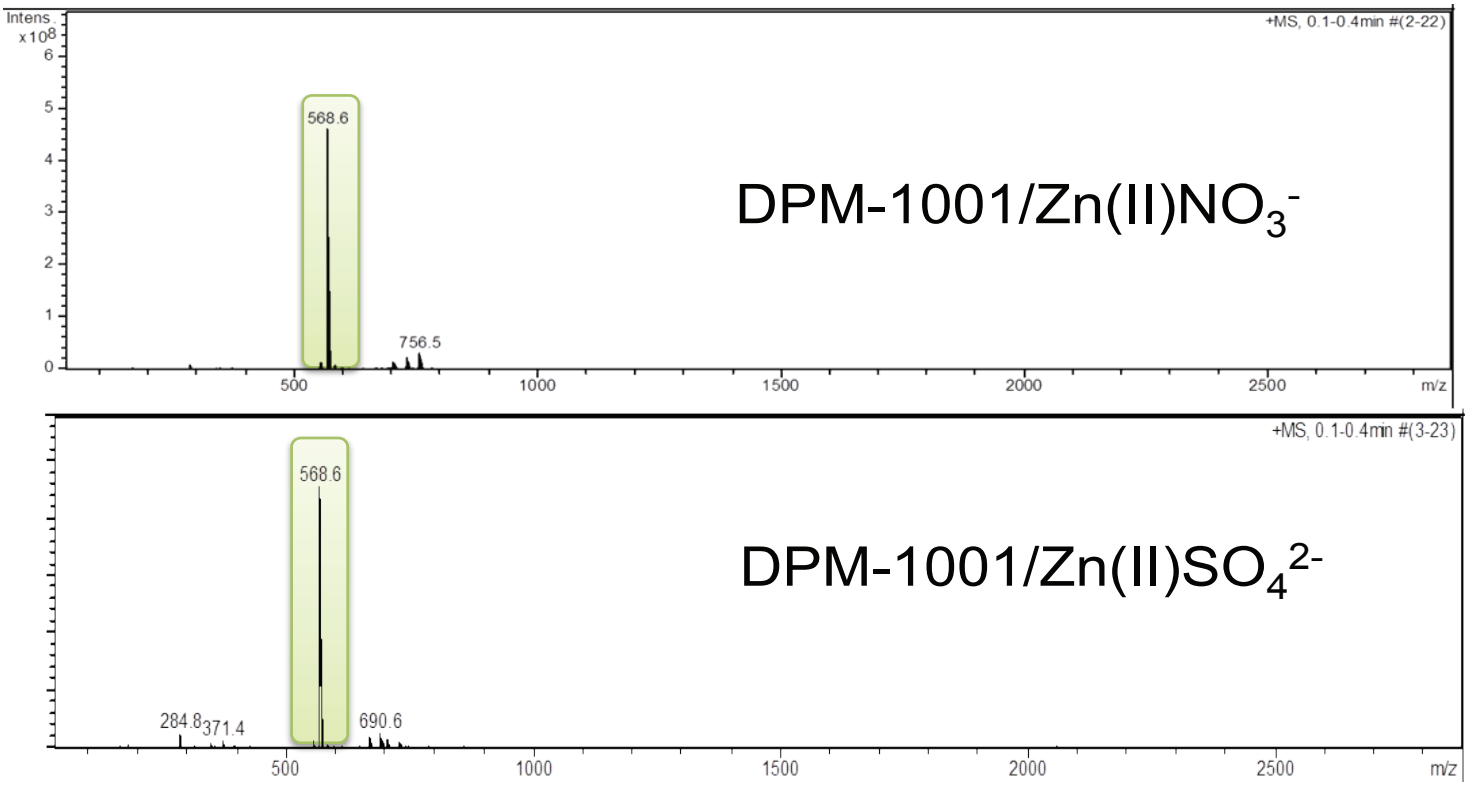

**B**



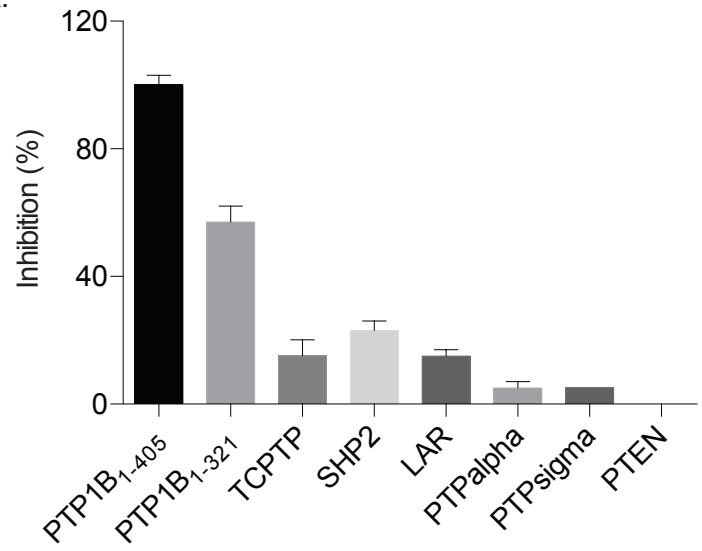

I



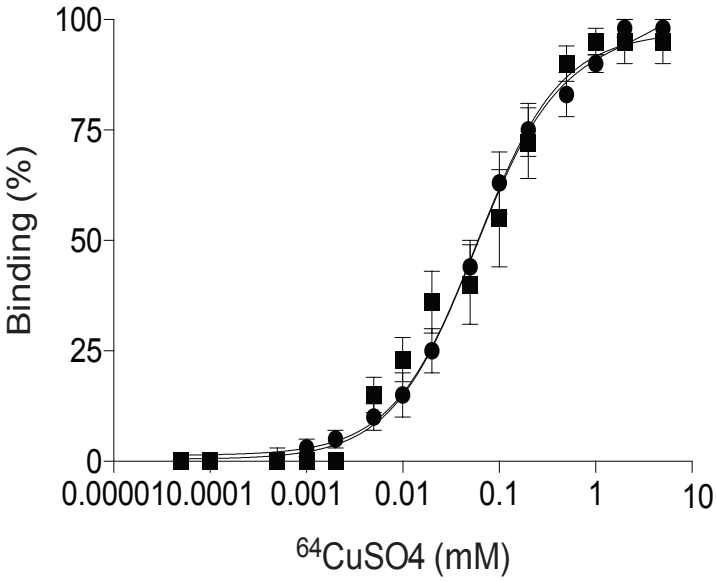

II


Intens
x10^8
+MS, 0.1-0.4min #(2-22)
568.6
756.5
DPM-1001/Zn(II)NO3-
m/z
+MS, 0.1-0.4min #(3-23)
568.6
284.8
371.4
690.6
DPM-1001/Zn(II)SO4 2-
m/z

A.


120
80
40
0
Inhibition (%)
PTP1B1-405
PTP1B1-321
TCPTP
SHP2
LAR
PTPalpha
PTPsigma
PTEN

B.


100
75
50
25
0
Binding (%)
0.00001
0.0001
0.001
0.01
0.1
1
10
64CuSO4 (mM)