Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Methods

Mass spectrometry analysis

The gel fragments containing the isolated proteins were destained, reduced in 10 mM DTT for 1 hour at 56ºC, and then alkylated in 55 mM chloroacetamide for 1 hour at room temperature. Gel fragments were washed in 50 mM ammonium bicarbonate and transferred to 100% acetonitrile. Protein digestion was performed using trypsin in 50 mM ammonium bicarbonate for 8 hours at 37ºC. The peptides were extracted in 90% acetonitrile and the sample dried in Speed Vac. Samples were resolubilized in a 5% acetonitrile, 0.2% formic acid solution. Samples were separated on a C18 column (150 μm × 10 cm) using an Eksigent nanoLC-2D system. A 56-min gradient from 5-60% acetonitrile (0.2% formic acid) was used to elute peptides from a reverse-phase column (150 μm i.d. x 100 mm) with a flow rate of 600 nL/min.

The column was connected to a nanoprobe interfaced with a Q-Exactive mass spectrometer (Thermo-Fisher). Each full MS spectrum was followed by 10 MS/MS spectra (eleven scan events), where the ten most abundant multiply charged ions were selected for MS/MS sequencing. Tandem MS experiments were performed using higher-energy collisional dissociation in the linear ion trap.

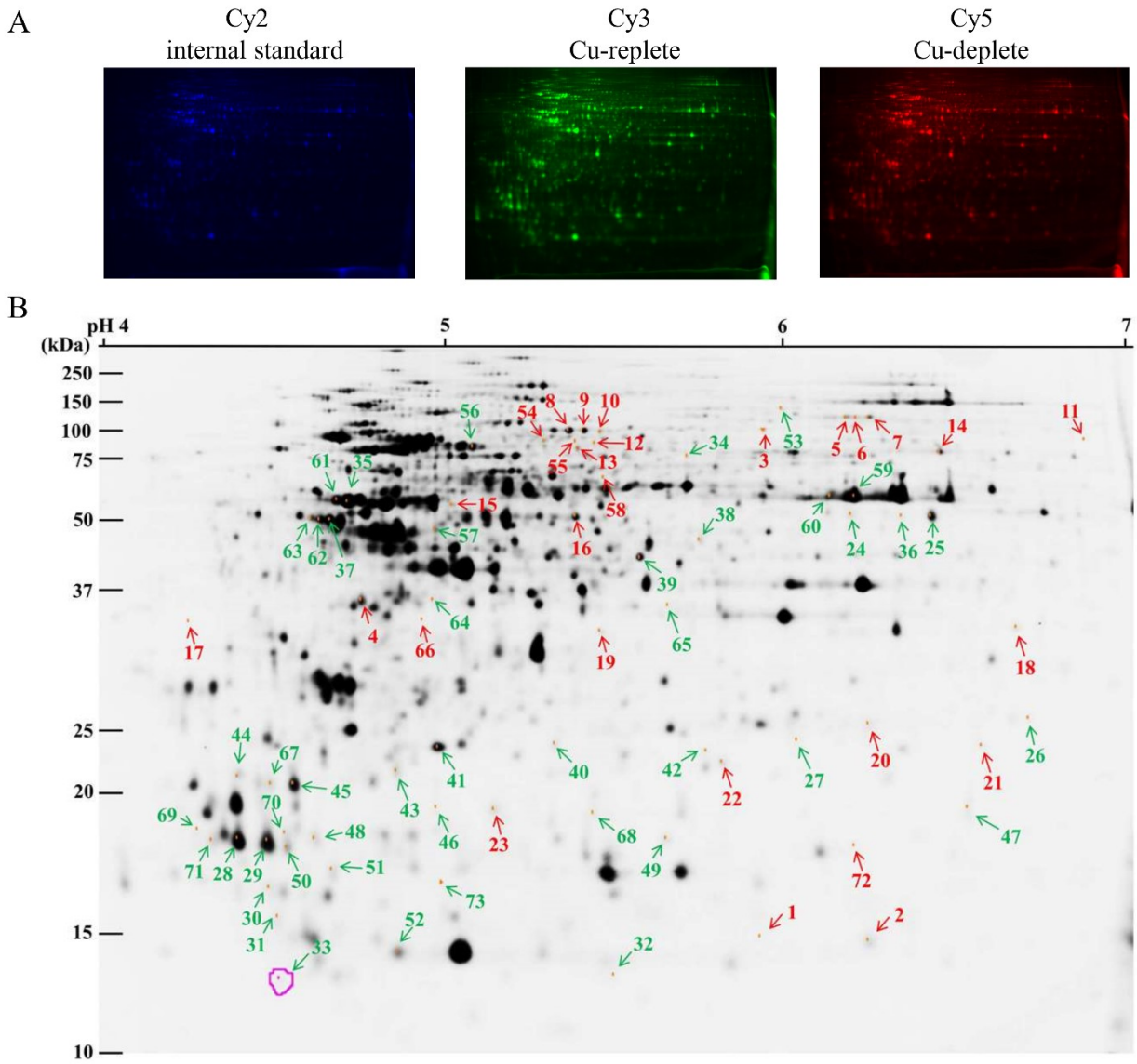

Figure S1. (A) 2D-DiGE images of proteins stained with Cy2, Cy3 or Cy5. (B) A representative internal standard gel image showing up-regulated (red) and down-regulated (green) proteins in response to Cu deficiency. Proteins were numbered sequentially during isolation.

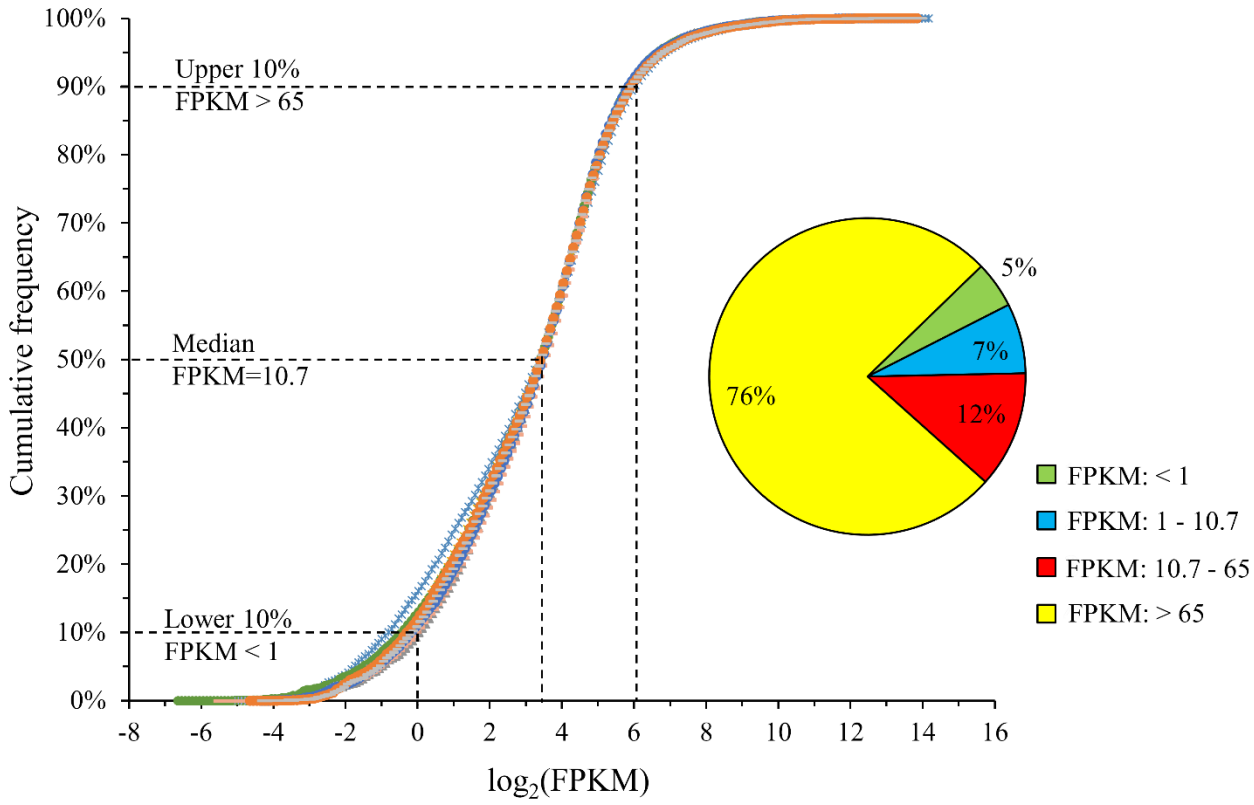

Figure S2. Cumulative frequency of transcripts of *T. oceanica* from low to high expression levels as a function of transcript expression. Transcript expression was quantified in an mRNA sequencing project and reported as values of Fragments Per Kilobase of transcript per Million (FPKM) mapped reads.[1] The pie chart shows the percentage of differentially expressed proteins identified in this study grouped according to level of transcript expression.

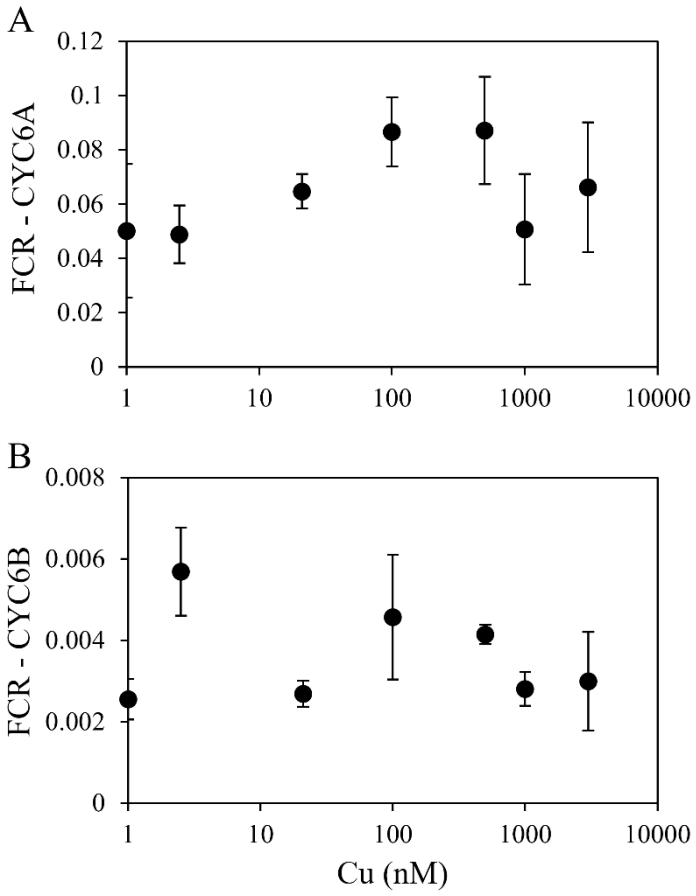

Figure S3. Relative expression of CYC6A (A) and CYC6B (B) in *T. oceanica* as a function of total Cu concentration in growth medium. Trace metals were added complexed to 100 μM EDTA, as described in the Materials and Methods. Fold-change ratio (FCR) was calculated by normalizing expression level of each gene to the diatom housekeeping gene, actin. Error bars represent ±1 standard deviation of three biological replicates.

References

1. L. Kong, Molecular and physiological responses of an oceanic diatom to copper deficiency, *PhD thesis*, McGill University, 2019.