Supplementary figure S1: Effect of copper and iron on growth and cellular copper contents. O. tauri cells were grown in modified f/2 medium containing either 10 μ M of the specific copper chelator BCS (-Cu) or 100 nM CuSO4 (+Cu) and (A) 100 nM or (B) 1 nM ferric citrate. After 7 days' cells were reinoculated into fresh media of the same composition. (C) Exponential growth rates were calculated for the growth period shaded in grey in (A) and (B). Data are presented as means ± SD from 4 independent experiments. Statistical analysis was performed using (A, B) two–way ANOVA or (C) student's t test. (D) Cellular copper contents in O. tauri were determined by ICP-MS following growth for 7 days in modified f/2 medium containing either 10 μ M of the specific copper chelator BCS or CuSO4 in the concentration indicated.

Supplementary figure S2: Functional categories associated with the genes upand downregulated in response to copper limitation. Pie charts represent the 18 functional categories defined in this work (see Table S1 for a detailed description)

Supplementary figure S3: Coomassie-staining of the polyacrylamide gel confirming equal loading of the immunoblot shown in Figure 1.

Supplementary figure S4: Alignment of ostta11g02300 with copper transporting P-type ATPase from A. thaliana chloroplast. Blue strips correspond to transmembrane helices. N-terminal MxCxxC metalbinding motifs and CPC ion-transduction motifs in a transmembrane domain are framed. The figure was generated with Geneious[®] 9.1.8 (Biomatters). Transmembrane regions were predicted with Transmembrane Prediction Tool Plugin.

Supplementary figure S5: effect of copper and iron on cellular (A) heme b and (B) chlorophyll a content in O. tauri O. tauri cells were grown in modified f/2 medium containing 1 nM or 100 nM ferric citrate and either 100 nM CuSO4 (+Cu) or 10 μ M of the specific copper chelator BCS (- Cu). Data are presented as means ± SD from 3 independent experiments. Statistical analysis was performed using student's t test with **p < 0.1 and ***p < 0.01.

Supplementary figure S6: Coomassie-staining of the polyacrylamide gel confirming equal loading of the immunoblot shown in Figure 3E.

Supplementary figure S7: Alignment of ostta17g01035 with cytochromes c6 and c6a. The conserved cysteine residues within the cytochrome c6a -specific additional loop are framed.