

Supplementary 1. LC50 values of treatments from different metal ions at 24 h and 96 h, intracellular Cu content, relative expression of Cu transporter genes in ZFL cells. The curve was generated by non-linear regression. (a) CuCl₂, 24 h. (b) CuCl₂, 96 h. (c) ZnCl₂, 24 h. (d) ZnCl₂, 96 h. (e) The intracellular Cu content of ZFL after 24 h CuCl₂ exposure. The curve was generated by linear regression. (f) The relative expression of Cu transporter genes related to *zAtp7b*. The bars represent the geometric mean of fold difference derived from biological replicates (n = 3) and error bars represent 95% confidence level. T-test revealed a significant Δ Ct difference (p > 0.05) between groups a, b, and c.

Supplementary 2. SWISS-MODEL-predicted Cu transporter structures with domain map and plasmid map. (a) zCtr1 (trimer, from 43th-184th AA). (b) zAtox1 (dimer, from 1st-65th AA). (c) zAtp7a (from 628st-1394th AA, without metal binding domain). (d) zAtp7b (from 537st-1289th AA, without metal binding domain). (e) Domain map of zCtr1, zAtox1, zAtp7a and zAtp7b. (f) Plasmid map of p(Cu transporter)-EGFP, pEGFP-(Cu transporter) and pcDNA3.1-(Cu transporter). CMV: cytomegalovirus promoter used in mammalian expression. SV40: simian vacuolating virus 40 promoter used in mammalian expression. AmpR: constitutive promoter used for expression in *Escherichia coli*. NeoR/KanR: aminoglycoside phosphotransferase expression for Kanamycin and G418 resistance. Ori: Origin of replication.

Supplementary 3. Protein sequence alignment of Ctr1 and Atox1 of different species by MUSCLE. (a) zCtr1: Ctr1 from *Danio rerio* (Q8QH07). zCtr1*: Ctr1 from this study. sCtr1: Ctr1 from *Sparus aurata* (Q700F3). hCTR1: CTR1 from *Homo sapiens* (O15431). rCTR1: CTR1 from *Rattus norvegicus* (Q9JK41). mCTR1: CTR1 from *Mus musculus* (Q8K211). (b) zAtox1: Atox1 from *Danio rerio* (E7FEZ9). zAtox1*: Atox1 from this study. sAtox1: Atox1 from *Sparus aurata* (Q53HX5). hATOX1: ATOX1 from *Homo sapiens* (O00244). rATOX1: ATOX1 from *Rattus norvegicus* (Q9WUC4). mATOX1: ATOX1 from *Mus musculus* (O08997).

Supplementary 4. Protein sequence alignment of Atp7a of different species by MUSCLE. zAtp7a: Atp7a from *Danio rerio* (Q4F8H5). zAtp7a*: Atp7a from this study. sAtp7a: Atp7a from *Sparus aurata* (D4N236). hATP7A: ATP7A from *Homo sapiens* (Q04656). rATP7A: ATP7A from *Rattus norvegicus* (P70705). mATP7A: ATP7A from *Mus musculus* (Q64430).

Supplementary 5. Protein sequence alignment of Atp7b of different species by MUSCLE. zAtp7b: Atp7b from *Danio rerio* (I3ITM6). zAtp7b*: Atp7b from this study. sAtp7b: Atp7b from *Sparus aurata* (D4N237). hATP7B: ATP7B from *Homo sapiens* (P35670). rATP7B: ATP7B from *Rattus norvegicus* (Q64535). mATP7B: ATP7B from *Mus musculus* (Q64446).

Supplementary 6. Protein sequence alignment of zAtp7a and zAtp7b by MUSCLE. zAtp7a*: Atp7a from this study. zAtp7b*: Atp7b from this study.