Supplementary Information

Metallothioneins regulate ATP7A trafficking and control cell viability during copper deficiency and excess

Nikita Gudekar[1,2], Vinit Shanbhag[2,3], Yanfang Wang[2,4,6], Martina Ralle[5], Gary A. Weisman[2,3], and Michael J. Petris[1,2,3,4,*]

[1]The Genetics Area Program, University of Missouri, Columbia, MO, 65211; [2]The Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, 65211; [3]The Department of Biochemistry, University of Missouri, Columbia, MO, 65211; [4]The Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO, 65211; [5]The Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, Oregon 97239.

[6]Present address: State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China, 100193.

Contents include

Supplementary Figure 1

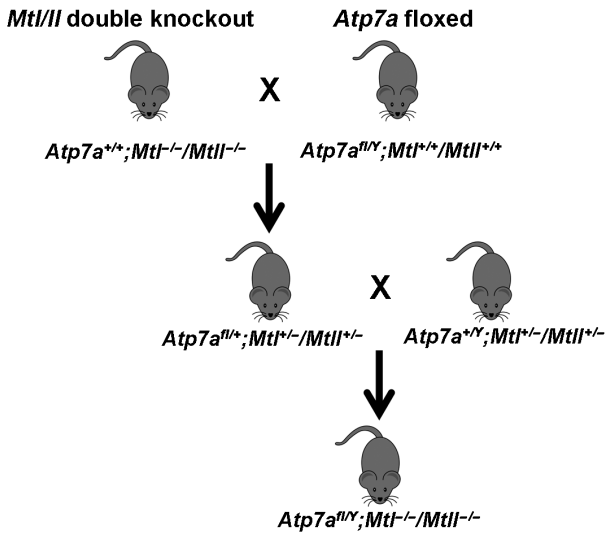

Fig S1: Breeding strategy used to generate *Atp7a* floxed mice carrying deletions in *Mtl* and *MtlI* genes. The *Mtl/II* knockout mice were crossed with *Atp7a* floxed mice to produce F1 offspring that were heterozygous at the *Mtl/II* locus and either Wt or floxed at the *Atp7a* locus. These mice were crossed to produce male *Atp7a* floxed mice that were homozygous null for the *Mtl/II* alleles.

Supplementary Figure 2

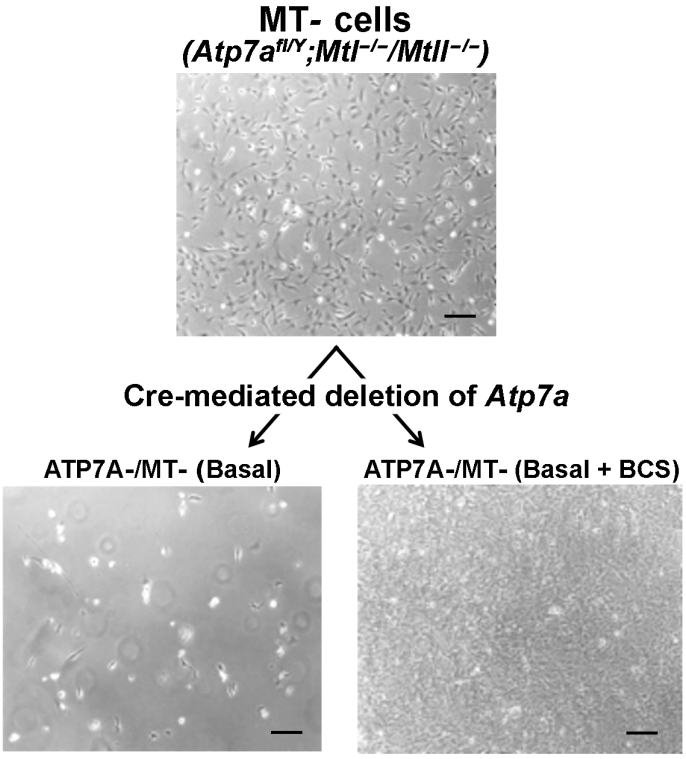

Fig S2: Deletion of the *Atp7a* gene in MT- cells causes a loss of cell viability that is rescued by Cu chelation. MT- cells were seeded overnight in basal medium at approximately 30% confluency (top panel), infected with an adenoviral Cre vector to delete the *Atp7a* gene and then cultured for 5 days in basal media with or without 50 μM BCS (bottom panels). Images were collected by light microscopy; bars, 50 μm.

Supplementary Figure 3

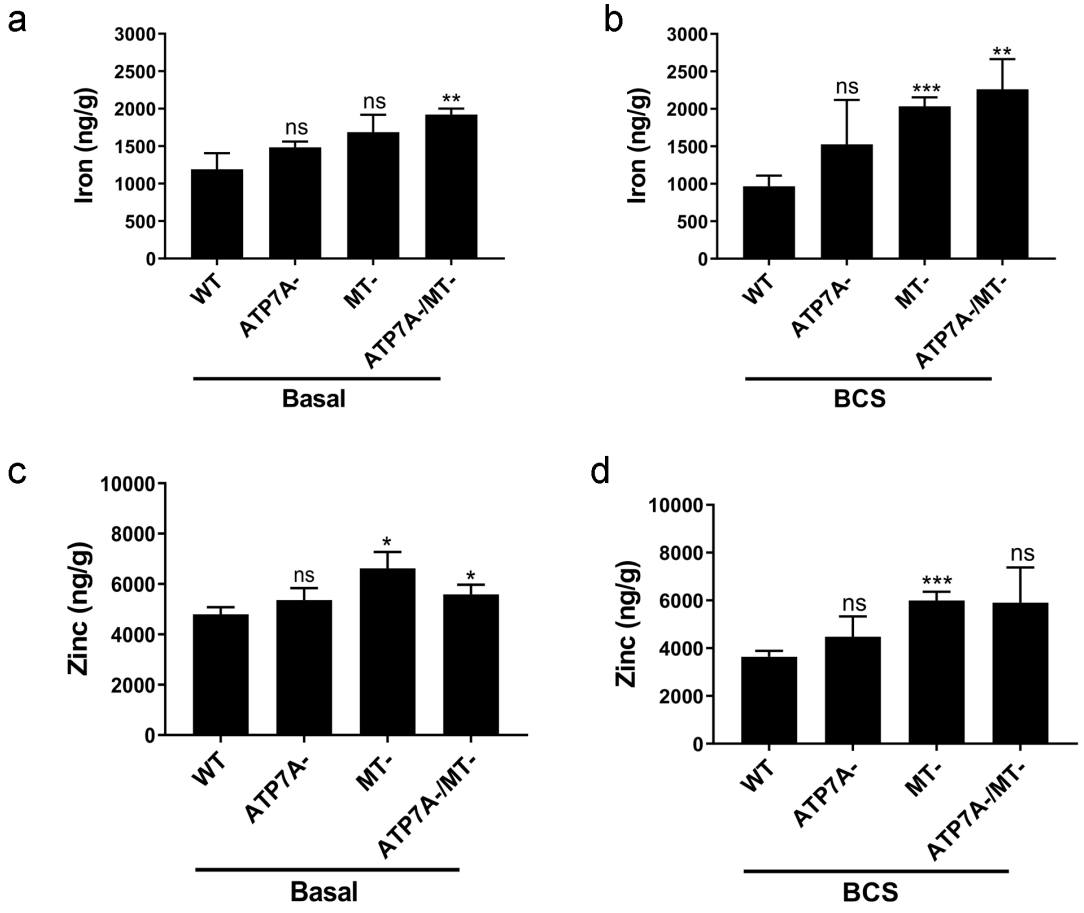

Fig S3: Iron (Fe) and Zinc (Zn) concentrations in each cell line were determined by ICP-MS. Cells were grown for 2 days in medium containing 50 μM of the copper chelator BCS and then exposed for 24 h to either basal media (basal) or media supplemented with 50 μM BCS. Values significantly different from WT are indicated (mean ± SEM; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns = not significant).

Supplementary Figure 4

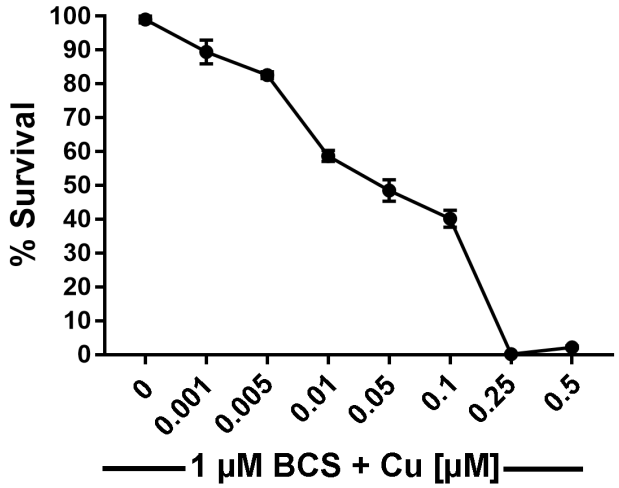

Fig S4: Deletion of ATP7A and MTI/II causes hypersensitivity to sub-micromolar Cu concentrations. ATP7A-/MT- cells were seeded into 6-well dishes (10^3 cells per well) and cultured for 6 days in media supplemented with 1 µM BCS with or without the indicated concentrations of $CuCl_2$. Surviving cells were quantified using the Crystal Violet assay (mean ± SEM).

Supplementary Figure 5

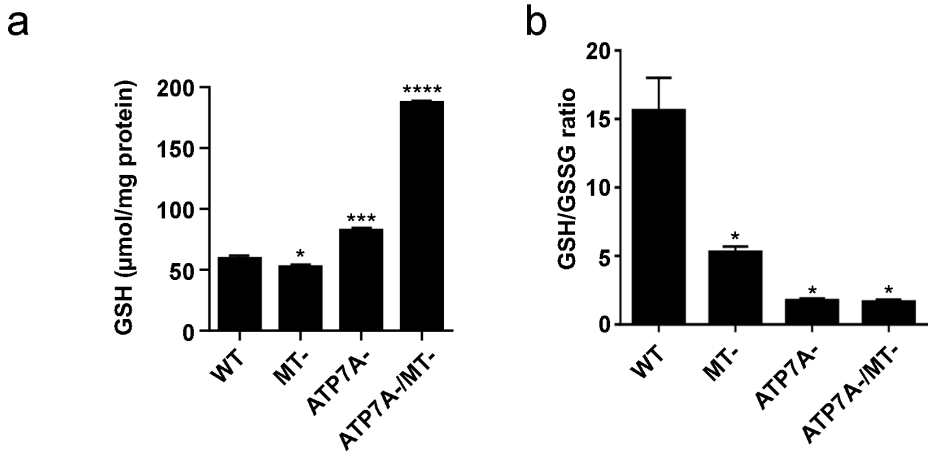

Fig S5: Deletion of ATP7A and MTI/II increases cellular GSH levels and reduces the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG). Cellular GSH per mg total protein (**a**) and the GSH/GSSG ratio (**b**) were measured in wild type (WT) and mutant cells cultured for 24 h in basal medium. Values are expressed as the mean ± SEM (*$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$; $n = 3$).

Supplementary Figure 6

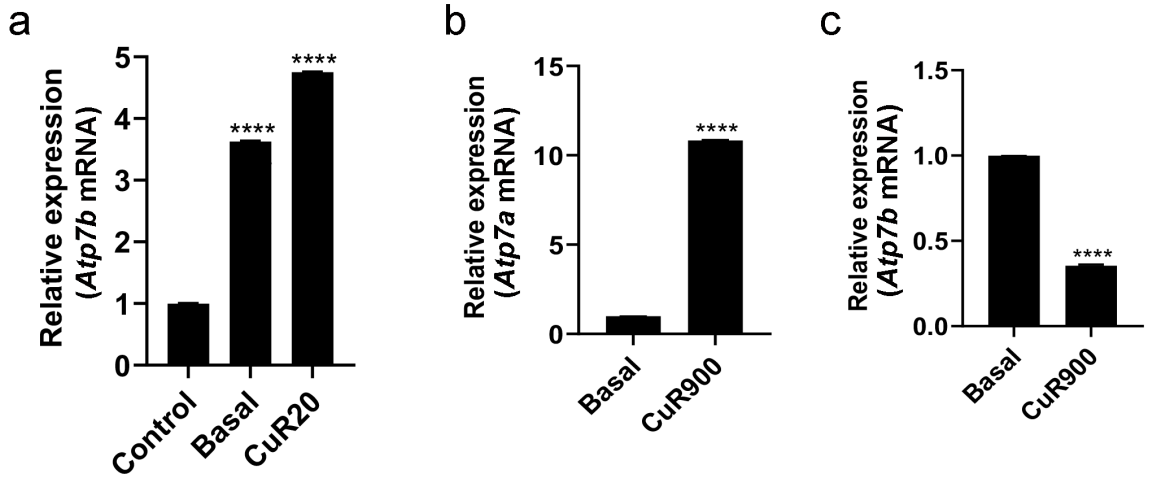

Fig S6: Quantitative RT-PCR analysis of *Atp7a* and *Atp7b* mRNA expression in spontaneously Cu resistant cell lines. (**a**) Parental ATP7A-/MT- (control) cells were selected in either basal medium (basal) or 20 μM $CuCl_2$ (CuR20). Quantitative RT-PCR was used to measure the abundance of *Atp7b* mRNA in each line normalized to *Gapdh* (mean ± SEM; ****p < 0.0001; n = 3). (**b and c**) Parental ATP7A+/MT- cells were grown in either basal medium (basal) or passaged in media containing elevated $CuCl_2$ until resistance to 900 μM was acheived (CuR900). Quantitative RT-PCR was used to measure the abundance of *Atp7a* and *Atp7b* mRNA in each line normalized to *Gapdh* (mean ± SEM; ****p < 0.0001; n = 3).

Full length gels and immunoblot from Figure 1

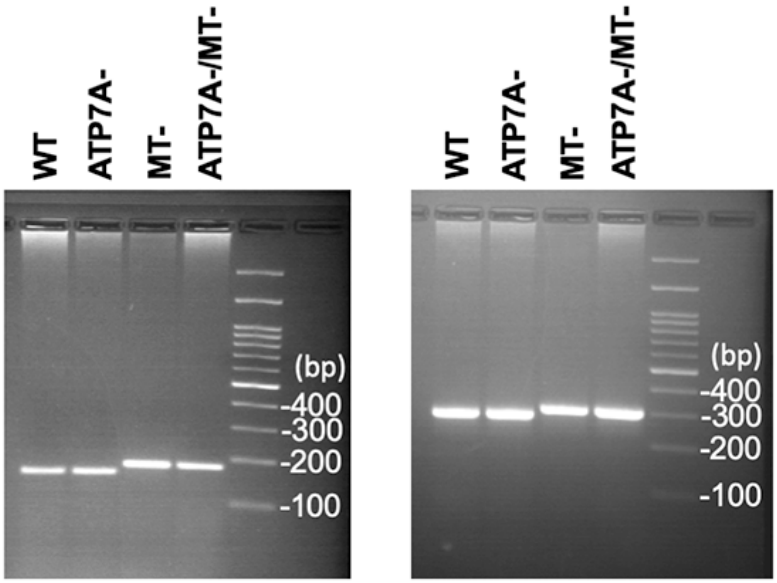

Fig. 1b

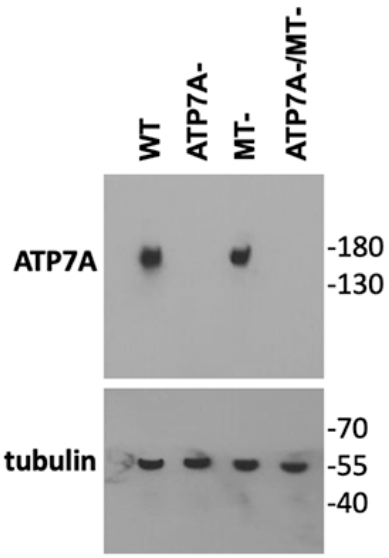

Fig. 1c

Full length immunoblots from Figure 4

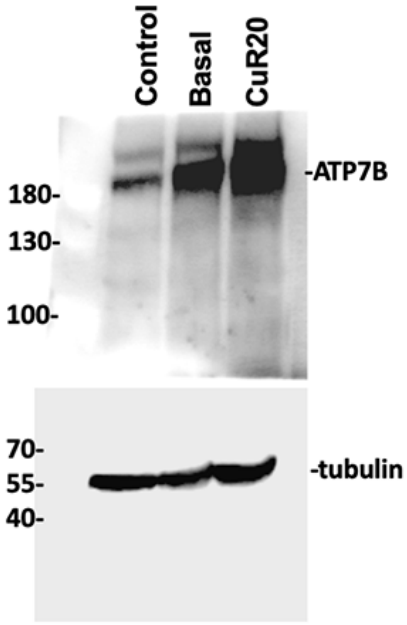

Fig. 4a

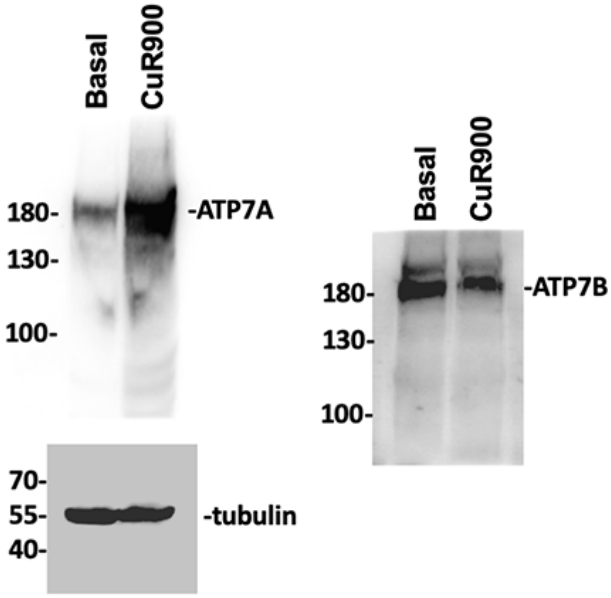

Fig. 4c