Electronic Supplementary Material (ESI) for Metallomics.

SUPPLEMENTAL INFORMATION

Probing functional roles of Wilson disease protein (ATP7B)

copper-binding domains in yeast

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Content:

Table S1. DNA Primers used in this study.

Name	Sequence (5'-3')
CCC2 Upstream Forward	GCGGCCAAGGAGTTGAGGTGTTTAATACTTAAAACGAAATGGGTAAG GAAAAGACTCACG
CCC2 Upstream Reverse	GCTTCAGCTGGCGGCCGCGTTCGTTTTAAGTATTAAACACCTCAAC
Atx1 Upstream Forward	CCTTCCATTTGTCCCAGGAGCGTAAATCTTTGGAATCC
Atx1 Upstream Reverse	GTCGACCGCCGGCGCATGAAAGTGAAGAGAAATAG
CCC2 Downstream Forward	GCGGCCGCGGATCTGCCGGATAACAGGTAAGTCTAATTTAT
CCC2 Downstream Reverse	CCTCAGTCAACTCTGGCAGAACCTCAGGAGGC
Atx1 Downstream Forward	GCCTAGACGGCCAGAGGGCTTTTGCGCTAGTTCTCTCTTGTGTCG
Atx1 Downstream Reverse	CCGAAAATTTGGGTGAGCTCGTATCTTTATTAGTGAACAACACCG
Kanamycin CCC2 Forward	GTTTAATACTTAAAACGAACGCGGCCGCCAGCTGAAGCTTCGTACGC
Kanamycin CCC2 Reverse	AGACTTACCTGTTATCCGGCAGATCCGCGGCCGCATAGGC
Kanamycin Atx1 Forward	CTCTTCACTTTCATGCGCCGGCGGTCGACTTCGAAGC
Kanamycin Atx1 Reverse	GAACTAGCGCAAAAGCCCTCTGGCCGTCTAGGCGCC
ATP7B Forward	GCCGGATCCAACAAAATGCCTGAGCAGGAGAGACAGATCAC
ATP7B Reverse	GCCGTCGACTCAGATGTACTGCTCCTCATCCCT
Atox1 Forward	GCCGGATCCAACAAAATGCCGAAGCACGAGTTCTCTGTGG
Atox1 Reverse	GGCGAATTCCTACTCAAGGCCAAGGTAGGAAACAGTCTTTCC
ATP7B 1DEL Forward	GCCGGATCCAACAAAATGGCAGAAGGAAAGGCAGCC
ATP7B 1-2DEL Forward	GCCGGATCCAACAAAATGAAGAGCAAAGTGGCTCCCTTAAGCC
ATP7B 1-3DEL Forward	GCCGGATCCAACAAAATGCCTGATGGAGCCGAAGGGAGTGG
ATP7B 1-4DEL Forward	GCCGGATCCAACAAAATGGTTTCTGAAAGCTGTTCTACTAACCC
ATP7B 1-5DEL Forward	GCCGGATCCAACAAAATGATGGAGGACTACGCAGGCTCCG
ATP7B 1-6DEL Forward	GCCGGATCCAACAAAGCCCAGAGAAACCCCAACGC
CPC-to-SPS Reverse	GGCCAGCCCCAGGGAGGAGGGGGAGGCAATGCACAG
CPC-to-SPS Forward	GTGCTGTGCATTGCCTCCCCCTCCTCCCTGGGGCTG
D1027A Reverse	GGGTAATGGTGCCAGTCTTGGCAAACATCACAGTC
D1027A Forward	CACAAGATAAAGACTGTGATGTTTGCCAAGACTGGCACC

Table S2. Summary of yeast strains constructed in this study and list of figures in which growth data is shown. Yeast strains with CCC2 gene, Atx1 gene, or both genes deleted are indicated as ΔCCC2, ΔAtx1, and ΔCCC2ΔAtx1. This information is followed by the names of the added high copy plasmids (containing Atox1 wild-type gene and ATP7B variants as indicated). ATP7B variants are explained in **Figure 1B**.

No	**Strain** (gene deletions and high copy plasmids)	**Growth data in Figure**
1	*S. cerevisiae* (Cen.pk 113.11C)	2
2	ΔCCC2	2,S2
3	ΔAtx1	2
4	ΔCCC2ΔAtx1	2,3,S1
5	ΔCCC2 + ATP7B	2,S2
6	ΔCCC2ΔAtx1 + ATP7B	2,S1
7	ΔAtx1 + Atox1	2
8	ΔCCC2ΔAtx1 + Atox1	2
9	ΔCCC2ΔAtx1 + ATP7B + Atox1	2,3
10	ΔCCC2 + ATP7B 1DEL	S2
11	ΔCCC2 + ATP7B 1-2DEL	S2
12	ΔCCC2 + ATP7B 1-3DEL	S2
13	ΔCCC2 + ATP7B 1-4DEL	S2
14	ΔCCC2 + ATP7B 1-5DEL	S2
15	ΔCCC2 + ATP7B 1-6DEL	S2
16	ΔCCC2 + ATP7B CPC-to-SPS	S2
17	ΔCCC2 + ATP7B D1027A	S2
18	ΔCCC2ΔAtx1 + ATP7B 1DEL + Atox1	3
19	ΔCCC2ΔAtx1 + ATP7B 1-2DEL + Atox1	3
20	ΔCCC2ΔAtx1 + ATP7B 1-3DEL + Atox1	3
21	ΔCCC2ΔAtx1 + ATP7B 1-4DEL + Atox1	3
22	ΔCCC2ΔAtx1 + ATP7B 1-5DEL + Atox1	3
23	ΔCCC2ΔAtx1 + ATP7B 1-6DEL + Atox1	3
24	ΔCCC2ΔAtx1 + ATP7B CPC-to-SPS + Atox1	3
25	ΔCCC2ΔAtx1 + ATP7B D1027A + Atox1	3
26	ΔCCC2ΔAtx1 + ATP7B 1DEL	S1
27	ΔCCC2ΔAtx1 + ATP7B 1-2DEL	S1
28	ΔCCC2ΔAtx1 + ATP7B 1-3DEL	S1
29	ΔCCC2ΔAtx1 + ATP7B 1-4DEL	S1
30	ΔCCC2ΔAtx1 + ATP7B 1-5DEL	S1
31	ΔCCC2ΔAtx1 + ATP7B 1-6DEL	S1
32	ΔCCC2ΔAtx1 + ATP7B CPC-to-SPS	S1
33	ΔCCC2ΔAtx1 + ATP7B D1027A	S1

Table S3. Growth rates (exponential phase) of modified yeast strains with ATP7B and Atox1 plasmids as indicated.

Strains	Growth rate (1/hr)
S. cerevisiae (Cen.pk 113.11c)	0.168±0.003
ΔCCC2	0.074±0.008
ΔCCC2+ATP7B	0.161±0.004
ΔAtx1	0.062±0.004
ΔAtx1+Atox1	0.150±0.002
ΔCCC2ΔAtx1	0.067±0.002
ΔCCC2ΔAtx1+ATP7B	0.068±0.001
ΔCCC2ΔAtx1+Atox1	0.071±0.002
ΔCCC2ΔAtx1+ATP7B+Atox1	0.164±0.003

Table S4. Growth rates of ATP7B variants (with and without Atox1) in the ΔCCC2 and ΔCCC2ΔAtx1 yeast strains.

Strains	Growth rate (1/hr)
ΔCCC2ΔAtx1	0.070±0.004
ΔCCC2ΔAtx1 + ATP7B	0.073±0.002
ΔCCC2ΔAtx1 + Atox1	0.075±0.004
ΔCCC2ΔAtx1 + ATP7B + Atox1	0.163±0.006
ΔCCC2ΔAtx1 + ATP7B 1DEL	0.073±0.010
ΔCCC2ΔAtx1 + ATP7B 1-2DEL	0.074±0.004
ΔCCC2ΔAtx1 + ATP7B 1-3DEL	0.077±0.005
ΔCCC2ΔAtx1 + ATP7B 1-4DEL	0.075±0.007
ΔCCC2ΔAtx1 + ATP7B 1-5DEL	0.076±0.002
ΔCCC2ΔAtx1 + ATP7B 1-6DEL	0.070±0.003
ΔCCC2ΔAtx1 + ATP7B CPC-to-SPS	0.071±0.008
ΔCCC2ΔAtx1 + ATP7B D1027A	0.070±0.004
ΔCCC2ΔAtx1 + ATP7B 1DEL + Atox1	0.097±0.002
ΔCCC2ΔAtx1 + ATP7B 1-2DEL + Atox1	0.103±0.004
ΔCCC2ΔAtx1 + ATP7B 1-3DEL + Atox1	0.155±0.006
ΔCCC2ΔAtx1 + ATP7B 1-4DEL + Atox1	0.141±0.005
ΔCCC2ΔAtx1 + ATP7B 1-5DEL + Atox1	0.147±0.004
ΔCCC2ΔAtx1 + ATP7B 1-6DEL + Atox1	0.075±0.003
ΔCCC2ΔAtx1 + ATP7B CPC-to-SPS + Atox1	0.071±0.004
ΔCCC2ΔAtx1 + ATP7B D1027A + Atox1	0.070±0.003
ΔCCC2	0.078±0.011
ΔCCC2 + ATP7B	0.156±0.005
ΔCCC2 + ATP7B 1DEL	0.104±0.004
ΔCCC2 + ATP7B 1-2DEL	0.115±0.012
ΔCCC2 + ATP7B 1-3DEL	0.154±0.004
ΔCCC2 + ATP7B 1-4DEL	0.147±0.006
ΔCCC2 + ATP7B 1-5DEL	0.149±0.007
ΔCCC2 + ATP7B 1-6DEL	0.062±0.008
ΔCCC2 + ATP7B CPC-to-SPS	0.068±0.003
ΔCCC2 + ATP7B D1027A	0.065±0.007

Figure S1. Western blot analysis of Atox1 and ATP7B expressed from high-copy plasmids in yeast strains with deletions of CCC2 and Atx1. A. *Expression of wild-type human proteins in ΔCCC2ΔAtx1 yeast strain.* Left. Gel analysis of purified Atox1 (from E. coli; control) and yeast sample grown under iron-limited conditions in ΔCCC2ΔAtx1 strain with high-copy plasmid containing Atox1 gene, using Atox1-specific antibody. Right. Gel analysis of wild-type ATP7B expressed in insect cells and purified into a membrane fraction (control) and yeast sample grown under iron-limited conditions in ΔCCC2ΔAtx1 strain with high-copy plasmid containing ATP7B gene, using ATP7B-specific antibody. **B.** *Expression of ATP7B variants in ΔCCC2ΔAtx1 yeast strain.* Gel bands stained with ATP7B antibody of yeast samples supplemented with high-copy plasmids for the different ATP7B variants as indicated. Whereas wild-type, CPC-to-SPS, and D1027A variants appeared at about 160 kDa molecular weight (according to marker bands), the domain deleted ATP7B bands shown appeared at molecular weights of decreasing size, roughly 150, 140, 130, 120, 110, and 100 kDa going for 1DEL to 1-6DEL. Because of difficulties getting the complete yeast samples into the gel, quantification of protein amounts was not performed.

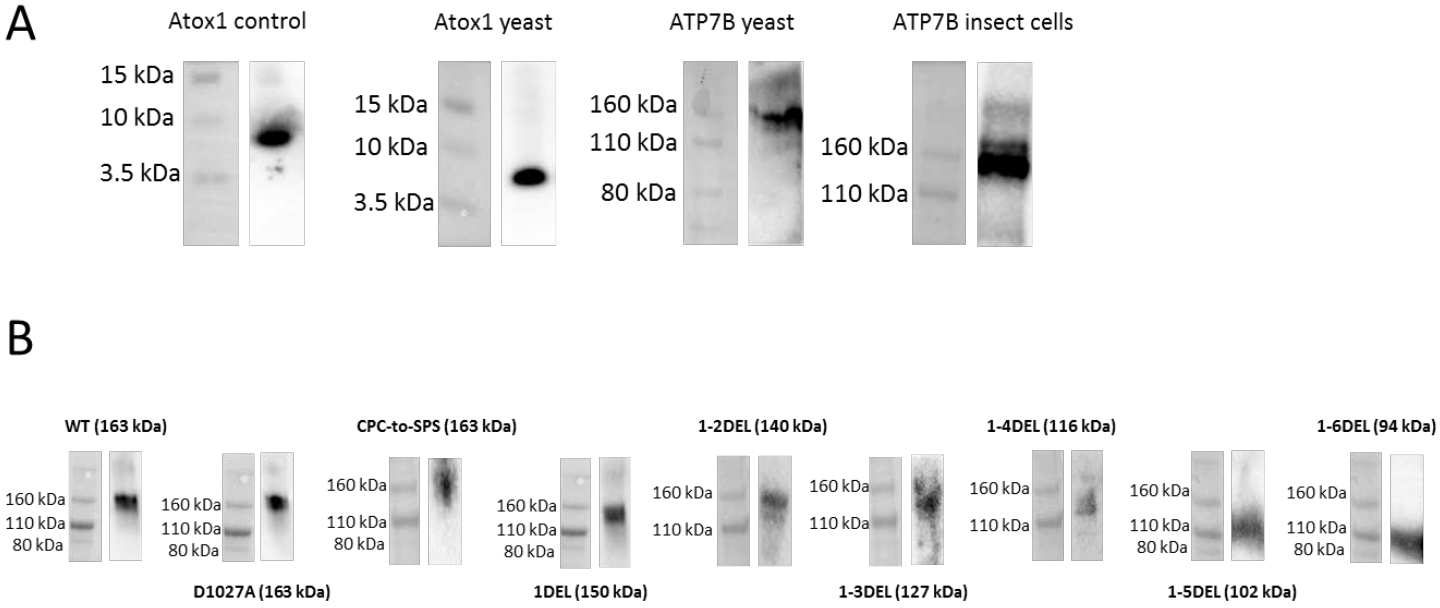

Figure S2. Examples of yeast growth curves, OD_{600nm} versus time, for various combinations of yeast strains and supplementation of high-copy plasmids with human Atox1 and/or ATP7B, as indicated, in iron-limited media

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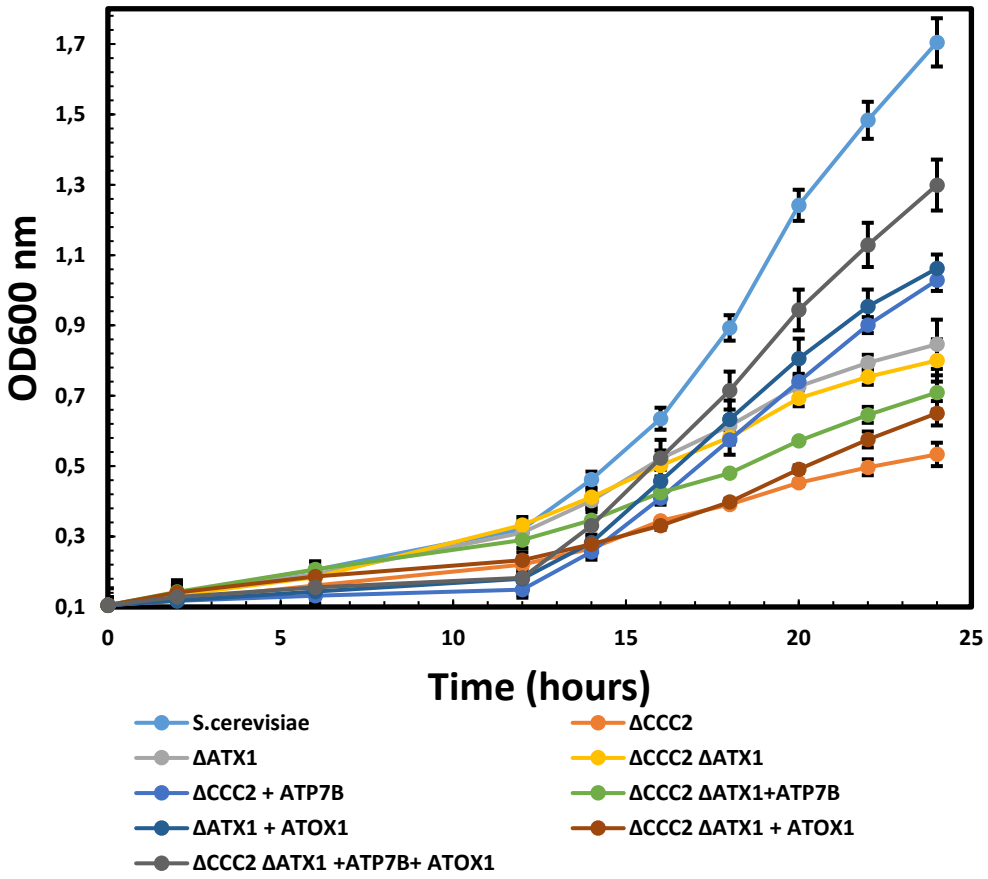

Figure S3. Growth rates (exponential phase) of various ATP7B variants supplied on plasmids in the ΔCCC2ΔAtx1yeast strain in the absence of the Atox1 plasmid in iron-limited media (for values, see **Table S4**). Error bars are based on standard deviation of duplicate measurements.

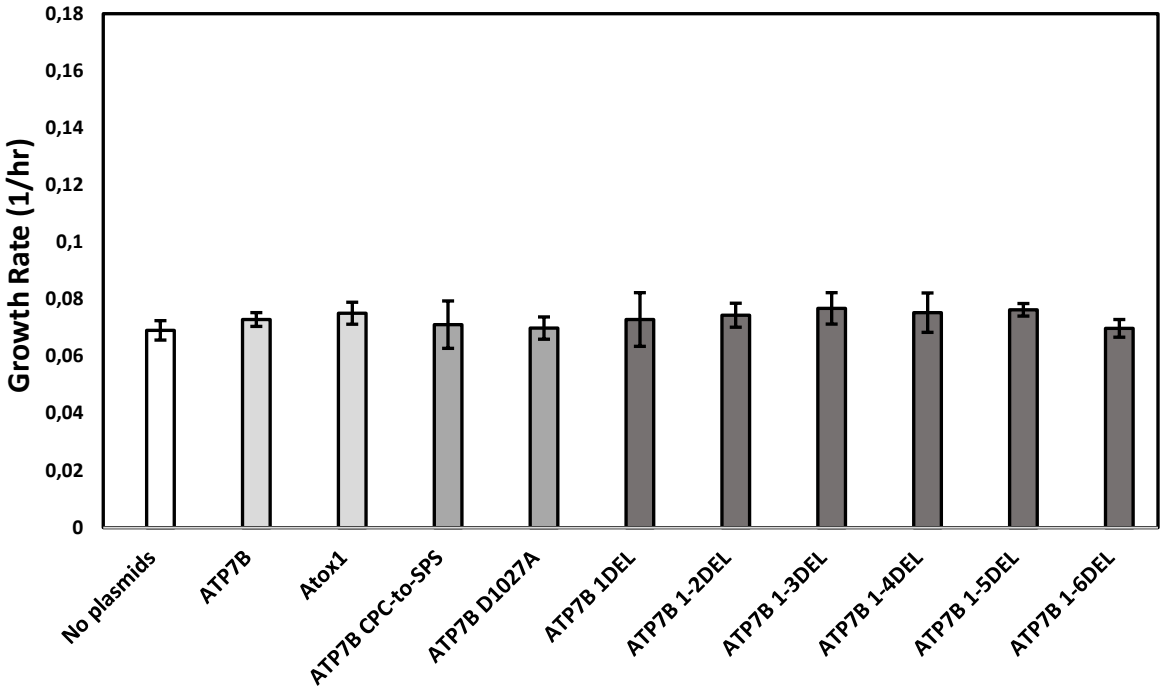

Figure S4. Growth rates (exponential phase) of various ATP7B variants supplied on plasmids in the ΔCCC2 yeast strain in the absence of the Atox1 plasmid (but here with endogenous Atx1 gene intact) in Fe-limited media (for values, see **Table S4**). Error bars are based on the standard deviation of duplicate measurements. Note that trends here for various MBD deletions follow the same trends as those in **Figure 3** (with human Atox1 instead of Atx1p).