

Supplemental Information

Copper relay path through N-terminus of Wilson disease protein, ATP7B

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Table S1. DNA Primers used in this study (ATP7B Cu-site mutants)

Primer Name	Primers (5'-3')
1CS_For	ATCTTGGGCATGACTAGCCAGTCAAGTGTGAAGTCCATTG
1CS_Rev	CAATGGACTTCACACTTGACTGGCTAGTCATGCCCAAGAT
2CS_For	AGGGCATGACCAGCCAGTCCAGTGTCTCAGCTCC
2CS_Rev	GGAGCTGACACTGGACTGGCTGGTCATGCCCT
3CS_For	CTCCAAGTGAAGAATAGATGGAATGCATAGTAAGTCTAGCGTCTTGAATATT
3CS_Rev	AATATTCAAGACGCTAGACTTACTATGCATTCCATCTATTCTCAGTTGGAG
4CS_For	GCCGGCATGACCAGTGCATCCAGTGTCCATTCCA
4CS_Rev	TGGAATGGACACTGGATGCACTGGTCATGCCGGC
5CS_For	GATCAAAGGCATGACCAGTGCATCCAGTGTGTCTAACATAGA
5CS_Rev	TCTATGTTAGACACACTGGATGCACTGGTCATGCCTTTGATC
6CS_For	CAGGGATGACCAGCGCGTCCAGTGTCCACAAC
6CS_Rev	GTTGTGGACACTGGACGCGCTGGTCATCCCTG

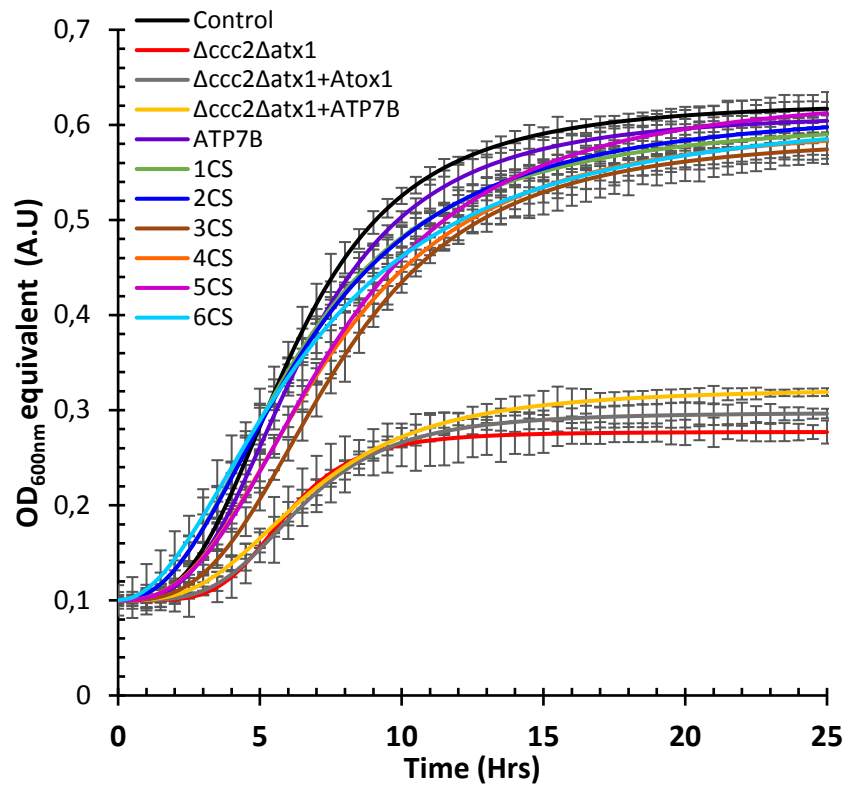
Table S2. DNA Primers used in this study (Individual MBD construct)

Primer Name	Primers (5'-3')
MBD1_For	GCCGGATCCAACAAAATGGCCACCAGCACAGTCAGGATCTTGGGC
MBD1_Rev	GGCGAATTCTTAAATGCTGGCCTCGAAGCCCATGTCCCC
MBD2_For	GCCGGATCCAACAAAATGGCTGTGGTCAAGCTCCGGGTGGA
MBD2_Rev	GGCGAATTCTTAGATGGCAGCTTCAAATCCCATGTCATTACATGGTCCC
MBD3_For	GCCGGATCCAACAAAATGGTCACCCTCCAAGTGAAGAATAGATGGAATGC
MBD3_Rev	GGCGAATTCTTAAAGAGAACTTTAAAATTCCCAGGTGGAAGTGCCTCG
MBD4_For	GCCGGATCCAACAAAATGAGTACCACTCTGATTGCCATTGCCGGCATGAC
MBD4_Rev	GGCGAATTCTTAGACTGAAGCCTCAAATCCCATGTCTTCTATAGCAGC
MBD5_For	GCCGGATCCAACAAAATGCAGAAGTGCTTCTTACAGATCAAAGGCATGACC
MBD5_Rev	GGCGAATTCTTAGACTGCTGCCTCAAAACCCAGGTCCTGGATGAAC
MBD6_For	GCCGGATCCAACAAAATGGGCAACATTGAGCTGACAATCACAGGGATG
MBD6_Rev	GGCGAATTCTTACAGGGAAGCATGAAAGCCAATTTCTC

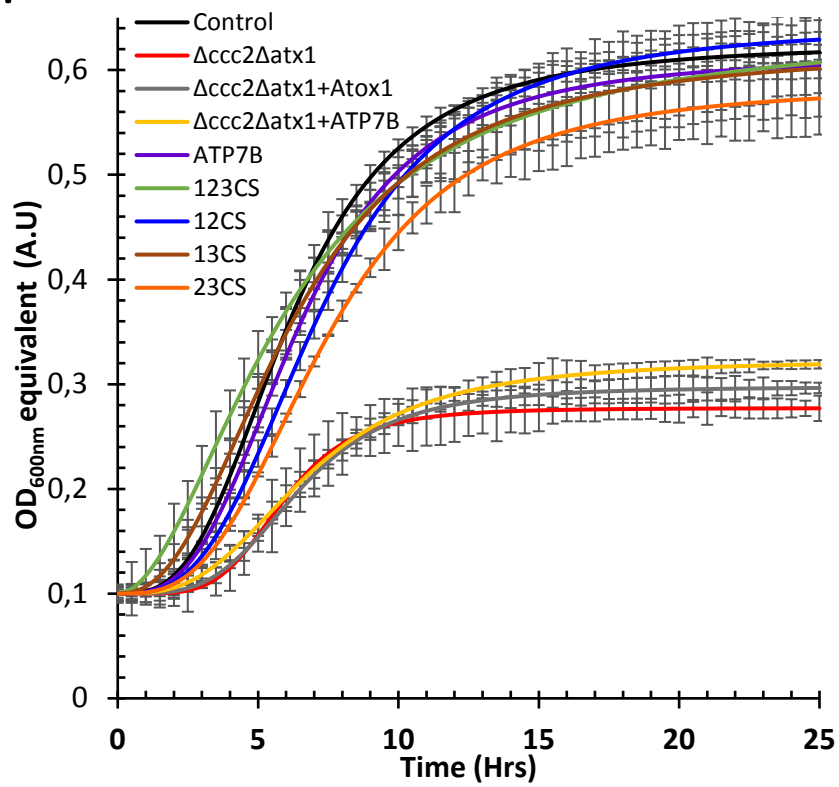
Figure S1. Growth curves of $\Delta ccc2\Delta atx1$ yeast with added plasmids for human proteins. (A-C) Growth curves (iron-limited conditions) of $\Delta ccc2\Delta atx1$ yeast carrying Atox1 and ATP7B (indicated variants) supplemented on high copy plasmids. **A.** ATP7B variants with individual MBD Cu sites mutated (1CS to 6CS). **B.** ATP7B variants with two or three MBD Cu sites mutated among MBD1-3. **C.** ATP7B variants with two or three MBD Cu sites mutated among MBD4-6. (D-F) Growth curves (iron-limited conditions) of $\Delta ccc2\Delta atx1$ yeast carrying ATP7B in combination with Atox1 or individual MBDs as chaperones supplemented on high copy plasmids. **D.** Atox1 or individual MBDs in combination with full-length ATP7B. **E.** Atox1 or individual MBDs in combination with ATP7B DEL1-3 (deletion of MBD1-3). **F.** Atox1 and individual MBDs in combination with ATP7B DEL1-6 (deletion of MBD1-6). Error bars are based on standard deviations calculated for quadruplicate experiments in four biological replicas. Analyses of exponential phases in this type of growth curves are the basis for growth rates reported in article figures.

Controls are: *S. cerevisiae* Cen.pk 113.11C without gene deletions as positive control; $\Delta ccc2\Delta atx1$ indicates yeast strain with CCC2 and ATX1 genes knocked out without added plasmids (negative control); $\Delta ccc2\Delta atx1$ + ATP7B corresponds to $\Delta ccc2\Delta atx1$ yeast with ATP7B without Atox1 plasmid; $\Delta ccc2\Delta atx1$ + Atox1 corresponds to $\Delta ccc2\Delta atx1$ yeast with Atox1 without ATP7B plasmid (both acting as additional negative controls). ATP7B indicates $\Delta ccc2\Delta atx1$ with wild-type ATP7B and Atox1 plasmids (another positive control).

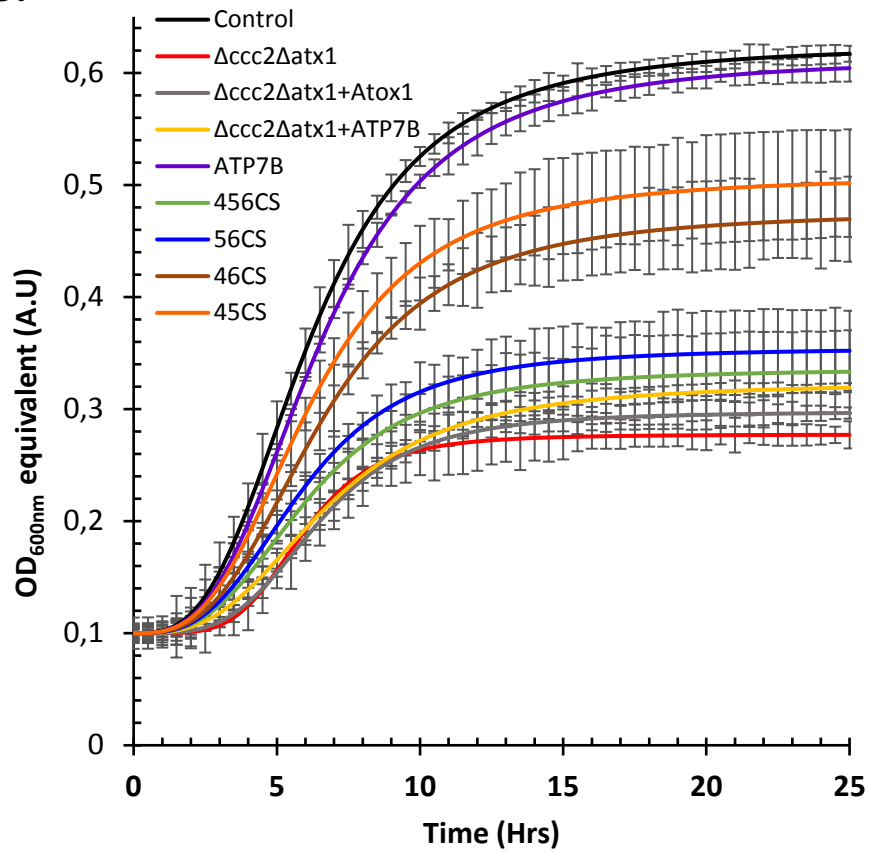
A.



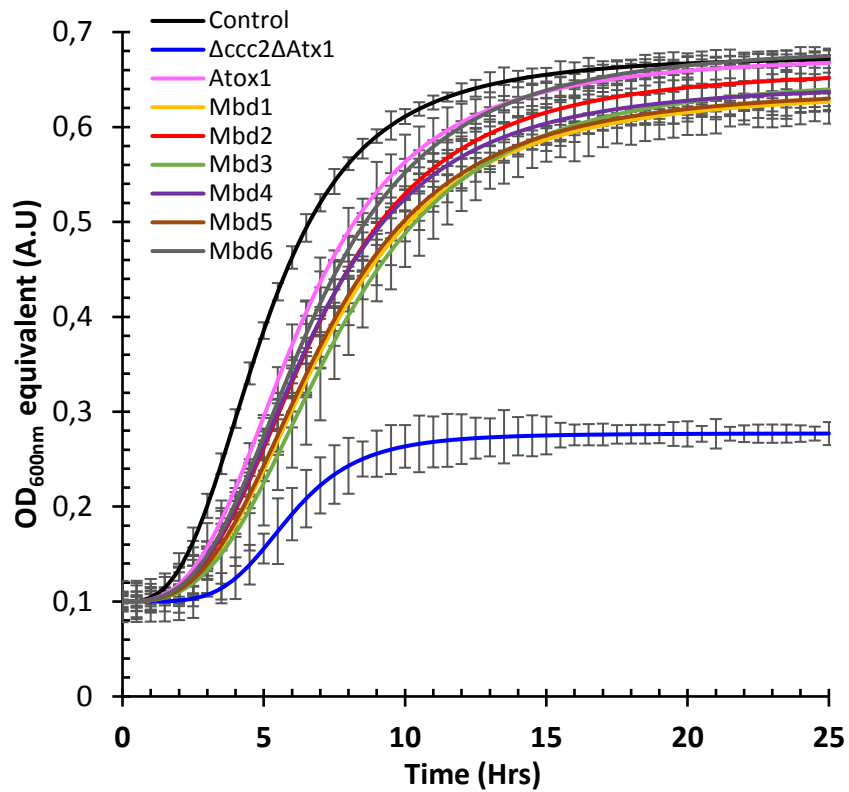
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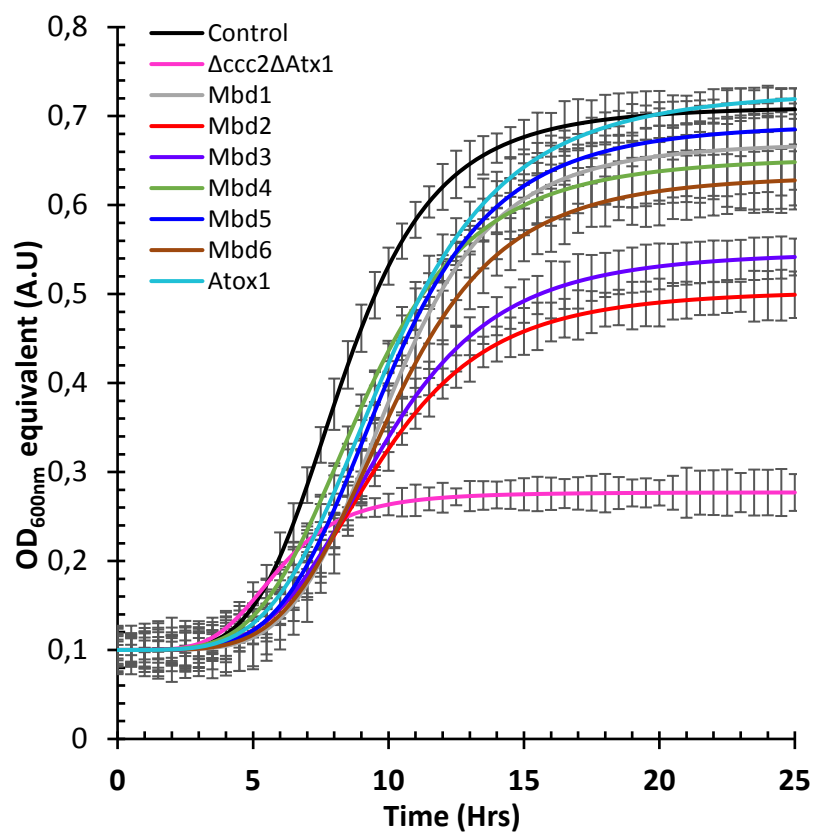
C.



D.



E.



F.

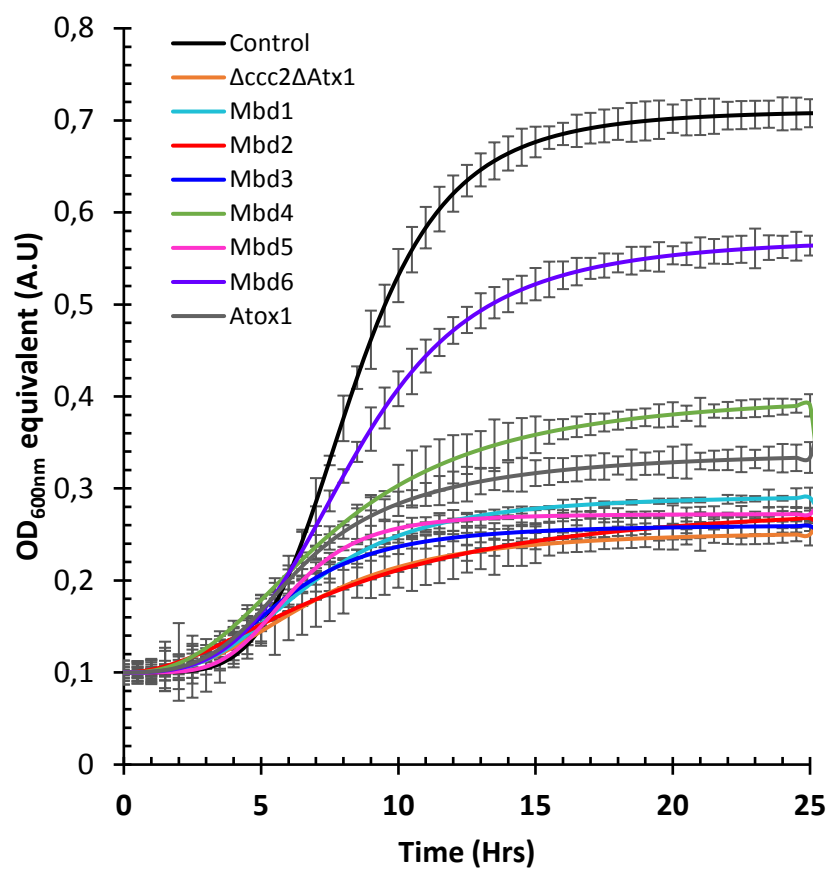
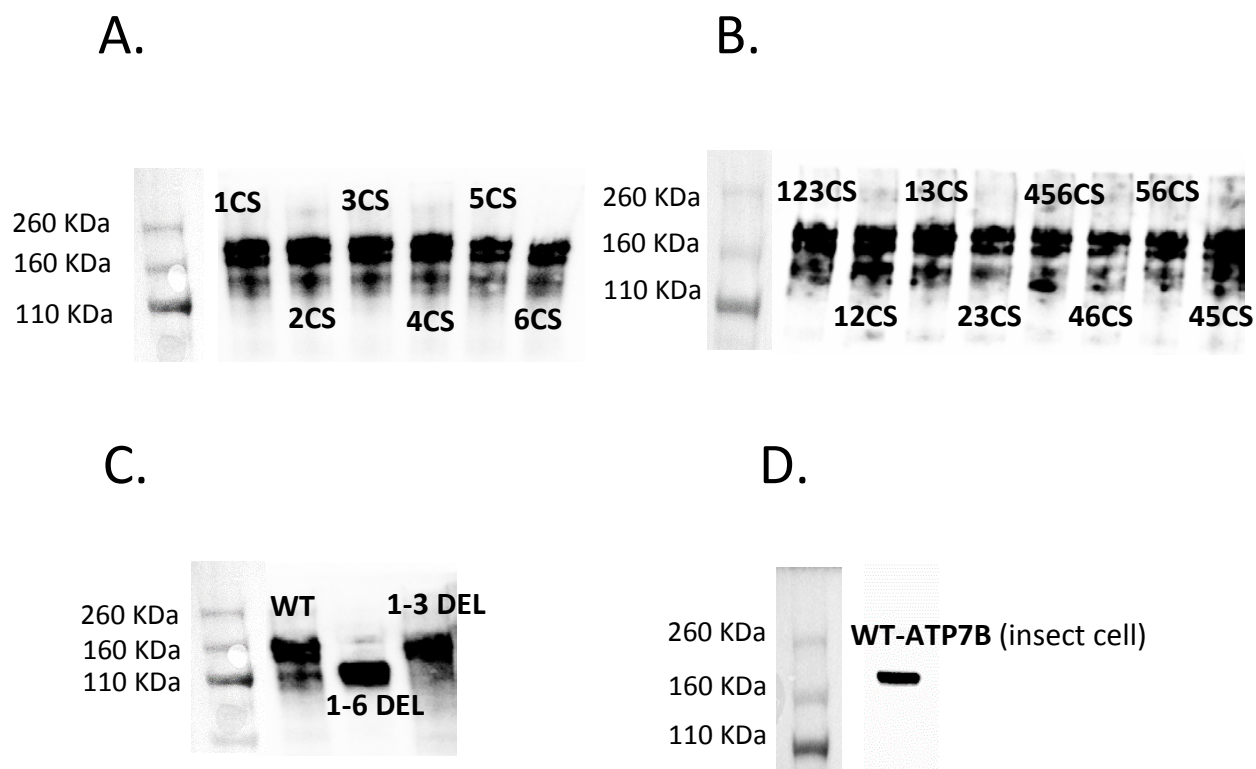


Figure S2. Western blot analysis of ATP7B variants expressed from high-copy plasmids in $\Delta ccc2\Delta atx1$ yeast strains using ATP7B specific antibody. **A.** ATP7B variants with individual Cu-site mutated as indicated. **B.** ATP7B variants with two or three Cu sites mutated as indicated. **C.** Metal-binding domains truncated ATP7B variants as indicated. **D.** Wildtype ATP7B expressed in insect cells¹ as control.



1. K. Ponnandai Shanmugavel, D. Petranovic and P. Wittung-Stafshede, Probing functional roles of Wilson disease protein (ATP7B) copper-binding domains in yeast, *Metallomics : integrated biometal science*, 2017, **9**, 981-988.