**Supplementary Table 1. Sequences of oligonucleotide primers used in this study, 5' to 3'**

| | | |
|---|---|---|
| HM | HM-fw | GCTGATGATACATATGATGGTTATGGACCA |
| | HM-rv | AAATTCATAGCTCTTCAGCATGGAAGAATCTGGCGCTC |
| Mut1 | Mut1fw | GGGGCTGATGATATATATGGGGGTTGCAGACGCAGGGTTTGC AACTCTTGCAGGGAATCAAAACATGAGTAAAGAAGAAAT |
| | Mut1rv | ATTTCTTCTTTACTCATGTTTTGATTCCCTGCAAGAGTTGCAA ACCCTGCGTCTGCAACCCCCATATATATCATCAGCCCC |
| Mut2 | Mut2fw | TTGCAACTCTTCACCATAATCAAAACGCAAGTAAAGAAGAAG CAATCAACCTTCATTCTTCTATGTTCC |
| | Mut2rv | GGAACATAGAAGAATGAAGGTTGATTGCTTCTTCTTTACTTGC GTTTTGATTATGGTGAAGAGTTGCAA |
| MBS-HM | T7-rv | TATGCTAGTTATTGCTCAG |
| | Intein-fw | CCCGCCGCTGCTTTTGCACGTGAG |
| | MBSHM-fw | GATGATCCGGGTGGAGGGCATGGACCACCACTTTGC |
| | MBSHM-rv1 | ACCGCCGAGCCCATAGAAGAATGAAGGTTGATCATTTCTTC |
| | MBSHM-rv2 | TTCAATGGAGCTGACCGCCGAGCCATAGAAGAATG |
| | MBSHM-rv3 | TCCGGACCTTGCCTTCAATGGAGCTGACCGCCGAGCCCATAG AAGAATGA |
| M52/56I-BScoHM | M52/56I-fw | ACTATATGTCCGCCAATCACCGCTCATATCACCGATCTGCAA AAAAAAC |
| | Xho-fw | GGCAGCTTCAATGACTCGAGGAAG-GGGATCCGGC |
| | HMBsco-rv | CATAGAAGAATGAAGGTTGATCATTTCTTCTTTACTCATGTT-TTGATTATGGTGAAGAGTTGCAAAGTGGTGGTGCATATTGGT AAAAATAAAATCCGC |
| | HMBsco-fw | ATGGACCACCACTTTGCAACTCTTCACCATAATCAAAACATG AGTAAAGAAGAAATGATCAACCTTCATTCTTCTATGCCGCCA ATCACCGCTCATATC |
| M52/56I-H55/135A-BScoHM | M52/56I-H55/135A-rv | TTTTTGCAGATCGGTGATAGCAGCGGTGATTGGCGGCAT |
| | M52/56I-H55/135A-fw | ATGCCGCCAATCACCGCT**GCT**ATCACCGATCTGCAAAA |
| ScoHM Loop mutant primers | Intein-rv | GGCACGATGTCGGCGATG |
| | ScoHMmut-fw1 | GATATAACCATGGGACAGCAGATTAAAGATCCGCTCAATTAC GAGGTGGA |
| | ScoHMmut-fw2 | ACGAGGTGGAGCCTTTTACATTTCAAAACCAAGACGGCAAGA ACGTTTCTTTA |
| | ScoHMmut-fw3 | CGTTTCTTTAGAGAGTTTAAAAGGAGAAGTATGGCTGGCGGA TTTTATTTTTA |
| ScoM12 | 2Metfw5 | TAATCAAAACATGAGTAAAGAAGAAATCATCAACCTTCATTC TTCTATCCCGC |
| | 2Metfw4 | TTTATTTTTACCAATATGGACCACCACTTTGCAACTCTTCACC ATAATCAAAC |
| ScoM1 | 1Metfw5 | TAATCAAAACATGAGTAAAGAAGAAATGATCAACCTTCATTC TTCTATCCCGC |
| ScoHis | 4Hisfw4 | TTTATTTTTACCAATATGGACGCCGGCTTTGCAACTCTTGCCG GTAATCAAAAC |

**Supplementary Table 2. Constructs used in this study (Location of His and Met residues in bold. Phe residue mutated to Trp for intrinsic fluorescence studies is shown in red).**

| Name of construct | HM-loop sequence and correlating amino acid number in wildtype ATP7A |
|---|---|
| ATP7A (MNK) | $^{672}$**M**V**M**D**HH**FATL**HH**NQN**M**SKEE**M**INL**H**SS**M**$^{699}$ |
| Mut1 | $^{672}$**G**V**A**D**AG**FATL**AG**NQN**M**SKEE**M**INL**H**SS**M**$^{699}$ |
| Mut2 | $^{672}$**M**V**M**D**HH**FATL**HH**NQN**A**SKEE**A**INL**H**SS**M**$^{699}$ |
| MBS2HM | $^{674}$**M**D**HH**FATL**HH**NQN**M**SKEE**M**INL**H**SS**M**$^{699}$ |
| ScoHM | $^{674}$**M**D**HH**FATL**HH**NQN**M**SKEE**M**INL**H**SS**M**$^{699}$ |
| ScoHis | $^{674}$**M**D**AG**FATL**AG**NQN**M**SKEE**M**INL**H**SS**M**$^{699}$ |
| ScoM1 | $^{674}$**M**D**HH**FATL**HH**NQN**I**SKEE**I**INL**H**SS**I**$^{699}$ |
| ScoM12 | $^{674}$**M**D**HH**FATL**HH**NQN**M**SKEE**I**INL**H**SS**I**$^{699}$ |

**Supplementary Figure 1**

Sequence of scaffold constructs used in this study with the inserted HM-loop sequence in green:

MBS2HM

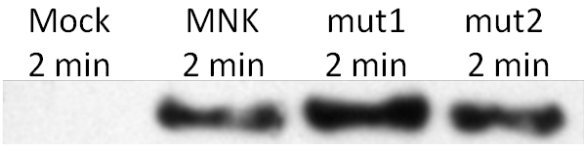
MQEAVVKLRVEG**MDHHFATLHHNQNMSKEEMINLHSSM**GSAVSSIEGKVRKLQGVVRVKVSLSNQEAVITYQPYLI
QPEDLRDHVNDMGFEAAIKS

ScoHM

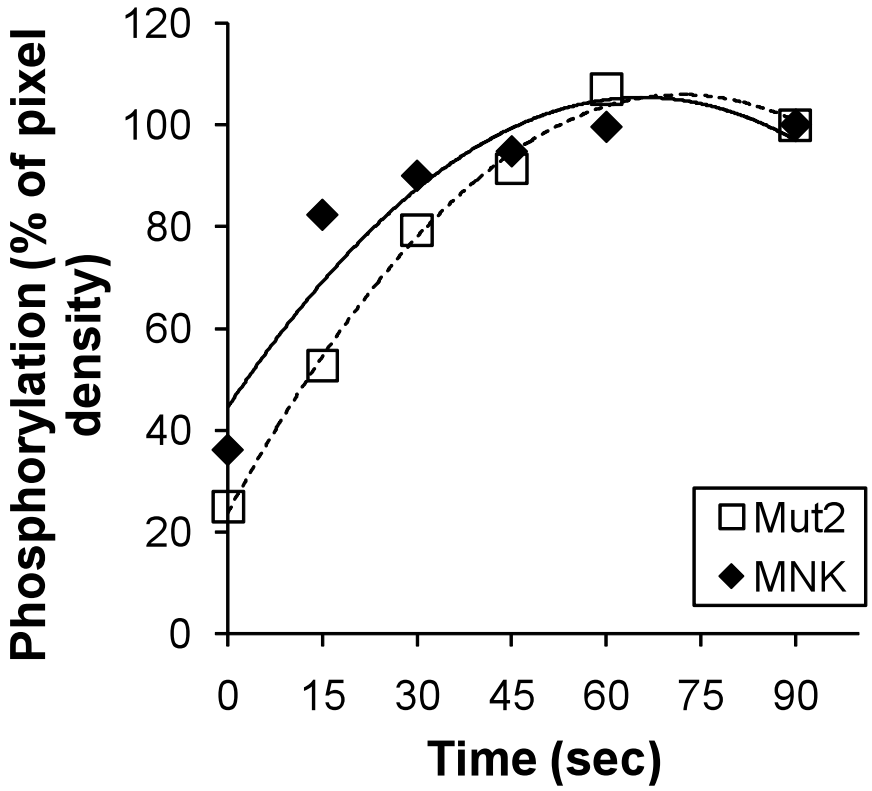
MGQQIKDPLNYEVEPFTFQNQDGKNVSLESLKGEVWLADFIFTN**MDHHFATLHHNQNMSKEEMINLHSSM**PPITAA
ITDLQKKLKAENIDVRIISFSVDPENDKPKQLKKFAANYPLSFDNWDFLTGYSQSEIEEFALKSFKAIVKKPEGEDQVIAQS
SFYLVGPDGKVLKDYNGVENTPYDDIISDVKSASTLK

**Supplementary Figure 2 Expression and characterization of ATP7A variants in insect Sf9 cells**
(A) Sf9 cells were infected with baculoviruses encoding the full-length ATP7A (MNK), ATP7A-Mutant 1 (mut1), and ATP7A-Mutant 2 (mut2). Western blot of SDS-PAGE-separated membrane fractions using antibody directed against the ATP7A C-terminus illustrates similar expression of all variants. No protein was detected in membranes from cells infected with empty virus, used as a control. (B) Example of time course of Mut2 phosphorylation compared to wildtype ATP7A (MNK).

**A**

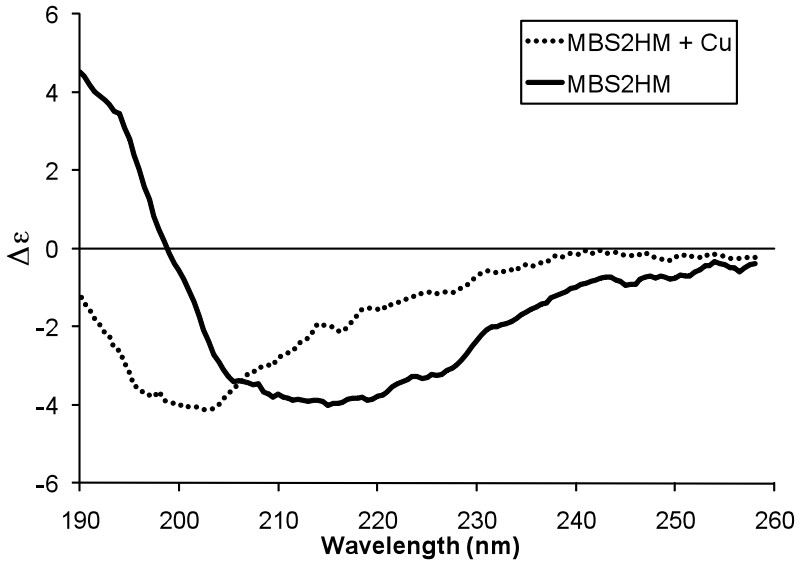



**B**



**Supplementary Figure 3 Copper binding to MBS2-HM induces significant structural change.**

Circular dichroism (CD) spectroscopy of purified recombinant MBS2-HM with and without addition of 1:2 mixture of $CuCl_2$ and glutathione, followed by dialysis. The decrease in α-helical content with simultaneous increase of the "random coil" signature" indicates that MBS-HM unfolds upon copper binding.



**Supplementary Figure 4. Optimization of the ascorbate content in the buffer for the BCS titration experiments.** Ascorbate was included into the buffer at increasing molear ratios to $CuCl_2$. BCS (1 ul o aliquots of 5 mM BCS solution) was then titrated to 500 ul solution containing 45 uM $CuCl_2$ in 50 mM sodium phosphate, pH 7.2. The formation of $Cu(I)(BCS)_2$ was measured at 483 nm using an absorption coefficient of 13500 $M^{-1} \cdot cm^{-1}$ and the amount of unchelated copper was calculated by subtracting complexed from total copper.