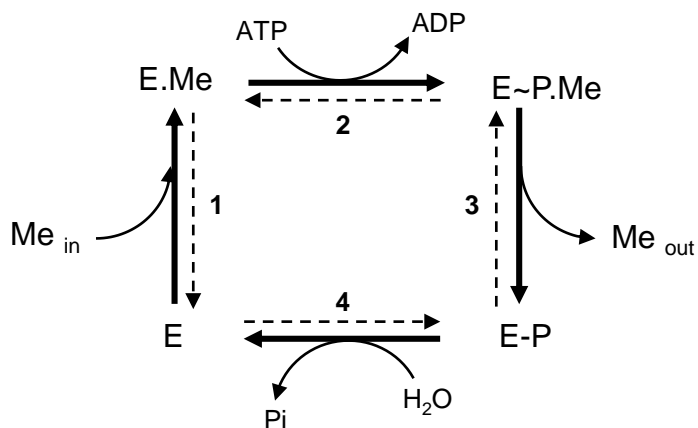


Supplemental Table 1. Bibliographic data of *in planta* characterizations of Arabidopsis *hma6/paa1*, *hma8/paa2* and *hma1* mutants.

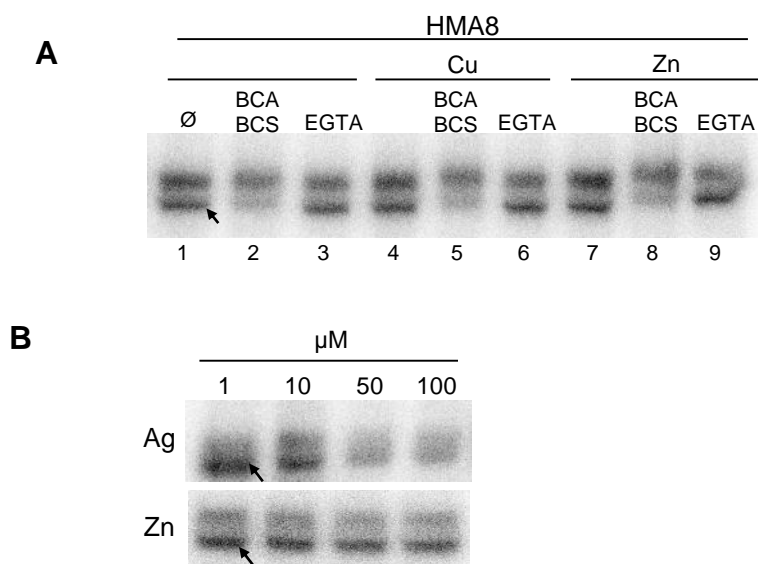
<p>Shikanai et al., 2003 [20]:</p> <p>Six independent mutants obtained by EMS in Arabidopsis Ler and Col ecotypes:</p> <ul style="list-style-type: none"> - Severe growth-rate phenotype partially rescued by addition of Cu. - High light-sensitivity. - Reduced Cu content and Cu/ZnSOD activity in chloroplasts from mutants. - Defect in photosynthetic electron transport, plastocyanin level are reduced drastically. <p>=> PAA1/HMA6 is a critical component of a Cu transport system in chloroplasts responsible for cofactor delivery to plastocyanin and Cu/ZnSOD.</p>
<p>Abdel-Ghany et al., 2005 [21]:</p> <p>Two independent mutants obtained by EMS in Arabidopsis Col ecotype:</p> <ul style="list-style-type: none"> - Pale green leaf color, growth rate unaffected. - Reduced Cu content in thylakoids. - Defect in photosynthetic electron transport, plastocyanin level are reduced drastically. <p>=> PAA2/HMA8 is required for Cu delivery to the thylakoid lumen.</p>
<p>Seigneurin-Berny et al., 2006 [22]:</p> <p>Two independent insertional mutants in Arabidopsis Ws ecotype:</p> <ul style="list-style-type: none"> - No phenotype in normal culture conditions. - High light-sensitivity, independent of Cu or Zn content. - Reduced Cu content and Cu/ZnSOD activity in chloroplasts from mutants. - In the chloroplast envelope from plants overexpressing AtHMA1, ATPase activity is enhanced by Cu. <p>=> AtHMA1 is an alternative pathway for Cu import into the chloroplast, essential in high light conditions</p>
<p>Kim et al., 2009 [25]</p> <p>Two independent insertional mutants in Arabidopsis Col-0 ecotype and one mutant in Ws ecotype:</p> <ul style="list-style-type: none"> - No phenotype in normal culture conditions. - Col-0 <i>hma1</i> mutants are more sensitive to excess Zn, accumulate more Zn in their shoots and chloroplasts. Chloroplast Cu concentration is not affected. <p>=> AtHMA1 contributes to Zn detoxification by reducing Zn concentrations in Arabidopsis plastids</p>
<p>Boutigny et al., 2014 [23]:</p> <p>Characterization of <i>paa1</i> mutant overexpressing HMA1 and <i>hma1 paa1</i> double mutant lines:</p> <ul style="list-style-type: none"> - Evidence for an alternative Cu import route in chloroplasts that is neither HMA1 nor PAA1. - The lack of HMA1 reinforces the phenotype of the <i>paa1</i> mutant (slower growth rate and decreased Cu/ZnSOD activity). - Overexpression of HMA1 in a <i>paa1</i> mutant background induces Cu-dependent photosensitivity. - HMA1 and PAA1 behave as distinct pathways for Cu import and targeting to the chloroplast. <p>=> New evidence for the role of AtHMA1 in chloroplast Cu homeostasis. Evidence for a third envelope transporter involved in regulating chloroplast Cu homeostasis.</p>

Supplemental Figure 1



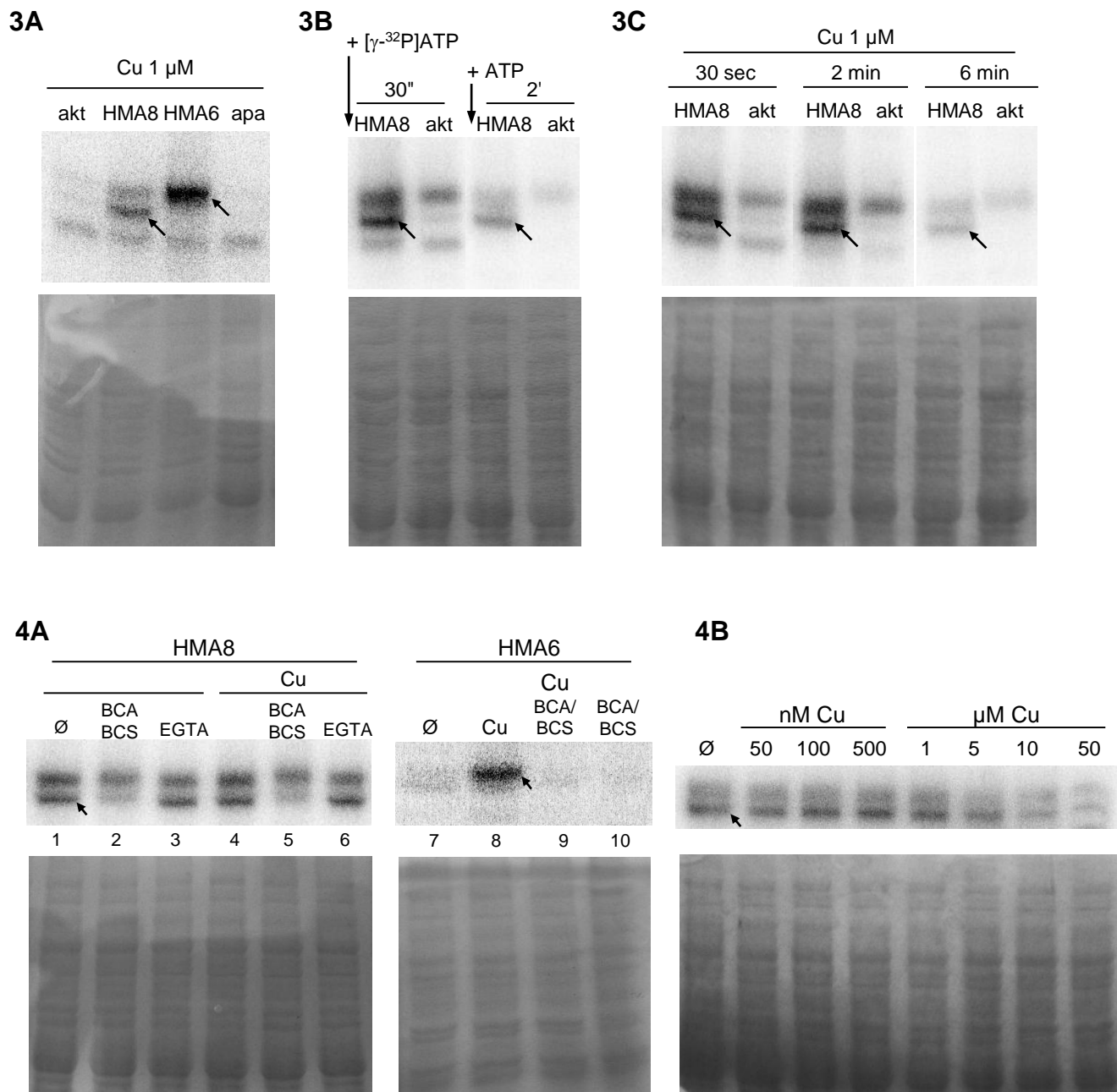
Supplemental Figure 1. Catalytic cycle of P_{IB}-type ATPases. The bold arrows correspond to the forward cycle of P-type ATPases. E, E.Me, E~P.Me, E-P represent the major catalytic intermediates of the enzyme. The four steps are reversible. Me represents the transported metal. Me_{in} and Me_{out} represent the cytosolic and extracellular/luminal metal respectively.

Supplemental Figure 2



Supplemental Figure 2. Metal dependence of HMA8 phosphorylation from [γ - ^{32}P]ATP. **A.** Membrane preparations containing HMA8 (150 μg) were phosphorylated without added metal (\emptyset , lanes 1 to 3), with 1 μM of CuSO_4 (lanes 4 to 6) or with 1 μM of ZnCl_2 (lanes 7 to 9). In each condition, BCA/BCS (lanes 2, 5 and 8) or EGTA (lanes 3, 6 and 9) was added as indicated in the figure. **B.** Membrane fractions (150 μg) containing HMA8 were incubated with various concentrations of AgNO_3 or ZnCl_2 . The arrows indicate HMA8 phosphorylation signals.

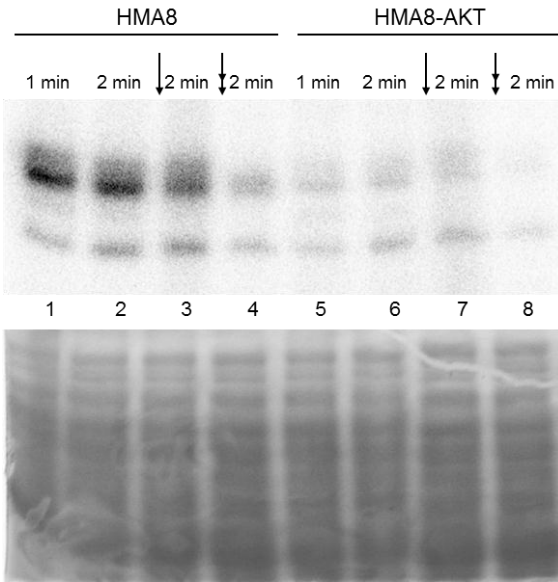
Supplemental Figure 3



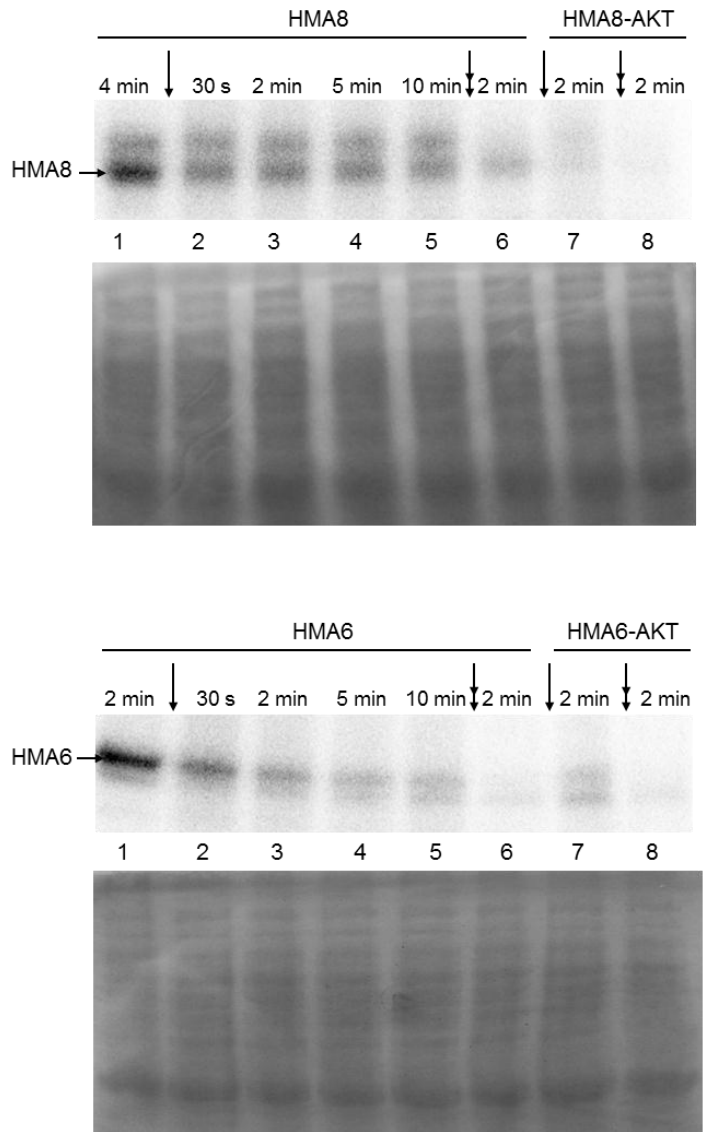
Supplemental Figure 3. Coomassie blue stained gels corresponding to the phosphorylation gels of the Figures 3 and 4. See Figures 3 and 4 for the legends.

Supplemental Figure 4

5B



6A



Supplemental Figure 4. Coomassie blue stained gels corresponding to the phosphorylation gels of the Figures 5B and 6A. See Figures 5B and 6A for the legends.