

Supplemental Material

Figure S1: Amino acid alignments of AtHMA2 and AtHMA3 according to ClustalW multiple alignment.

The transmembrane segments (TMD), Metal Binding Domain (MBD), HMA, and P-ATPase signatures are indicated. The arrow indicates the position of the base pair deletion in the gene encoding *AtHMA3*, which was observed in the Col-0 ecotype genome. This deletion induces a premature stop codon immediately downstream.

Figure S2: *AtHMA3* transcripts are enhanced in *35S::AtHMA3* and *35S::EGFP::AtHMA3* overexpressing plants.

A 630 bp fragment of the *AtHMA3* cDNA was amplified by RT-PCR, using total extracted RNAs from leaves of the various lines (wild-type Ws and overexpressing lines 35J, 36A, 27L, 38F, 13C, 18J, 19K and 27G). *Atactin 2* cDNA was used as control.

Figure S3: Root lengths of wild-type and various *35S::AtHMA3* and *35S::EGFP::AtHMA3* overexpressing lines under metal stress.

Experiments were performed as described in the legend of figure 3.

Movie 1: Confocal imaging in z-axis of a Ws protoplast, loaded with BTC-5N, as previously described in the legend of figure 5.

Movie 2: Confocal imaging in z-axis of a Ws protoplast loaded with BTC-5N after a 5 min 0.35 μ M Cd treatment.

Movie 3: Confocal imaging in z-axis of a *35S::AtHMA3* protoplast, loaded with BTC-5N, as previously described in the legend of figure 5.

Movie 4: Confocal imaging in z-axis of a *35S::AtHMA3* protoplast, loaded with BTC-5N, after a 5 min 0.35 μ M Cd treatment. The protoplast bursts at the end of the recording.