	_ MBD _
AtHMA3	MAEGEE <mark>SKKM</mark> NLQT <mark>SYFDVVGICCSSEV</mark> SIVG <mark>NVLRQVDGVKEFSVIVPSRTVIVVHDT</mark> FLI <mark>S</mark> PL <mark>QIVKALNQARLEASVR</mark> PY <mark>GETSLK</mark>
AtHMA2	MA <mark>SKKM</mark> TK <mark>SYFDVLGICCTSEV</mark> PLIENILNS <mark>MDGVKEFSVIVPSRTVIVVHDT</mark> LILS <mark>QFQIVKALNQAQLEANVR</mark> VT <mark>GETNFK</mark>
Athma3 Athma2	TMD1 QWPSPFATVSGVLLVLSFFKYF <mark>YSP</mark> LEWLATVAVVAGVFPILAKAVASVTRFRLDINALTLIAVIATLCMQDFTEAATIVFLFSVADWL KWPSPFAVVSGILLLLSFFKYLYSPFRWLAVAAVVAGIYPILAKAVASLARFRIDIN <mark>IL</mark> VVVT <mark>VGATIGMQDYTEAA</mark> VVVFLFTIAEWL
AtHMA3	S SAAHKAS I VM <mark>S S LMS LAPRKAVI AD TGLEVD VDEV</mark> G I NTV VS VKAGES I PIDG V VVDGS CD VDEKTLTGES FP V <mark>S KOREST VMAATIN</mark>
AtHMA2	S RASYKAS A VMO <mark>S LMS LAPOKAVI AE TGEEVEVDEL</mark> KT <mark>NTVI AVKAGET I PIDG V V VDGN CEVDEKTLTGEA FP V</mark> PKLKD STVWAGT I N
AtHMA3 AtHMA2	NGYIK <mark>VKTTALA</mark> RDCVVAKMTKLVEEAQKSQTKTQRFIDKCSRYYTPAVVVSAACFAVIPVLLKVQDLSHWFHLALVVLVSGCPCGLIL NGYITVNTTALAEDCVVAKMAKLVEEAQNSKTETQRFIDKCSKYYTPAIILISICFVAIPFALKVHNLKHWVHLALVVLVSACPCGLIL HMA signature
Athma3	TPVATFCALTKAATSGFLIKTGDCLETLAKIKIVAFDKTGTITKAEFMVSDFRSLSPSINLHKLLNWVSSIECKSSHPMAAALIDYAIS
Athma2	TPVATFCALTKAATSGLIIKGADYLETLAKIKIVAFDKTGTITRGEFIVMDFQSLSEDISLQSLLYWVSSTESKSSHPMAAAVVDYARS
AtHMA3 AtHMA2	SVEPKPDI <mark>ven Fon Fpgegvygridgod i yignkria</mark> oragoltdn <mark>vpdie</mark> atm <mark>krgktigyiymgakl</mark> tgsfnlldgorygvaoalke Svepkpeavedyon Fpgegiygkidgkevyignkria <mark>sragols vpdid</mark> vdtkggktigyvyvgetlagvfnlsdacrsgvaoamke Stop
AtHMA3	KSLGIQTAMLTGDNQDAAMSTQEQLENALDIVHSELLPQDKARIIDDFK IQGPTMMVGDGLNDAPALAKADIGISMGISGSALATETG
AtHMA2	KSLGIKIAMLTGDNHAAAMHAQEQLGNAMDIVRAELLPEDKSEIIKQLKREEGPTAMVGDGLNDAPALATADIGISMGVSGSALATETG
AtHMA3	IIILMSNDIRKIPKGMRLAKRSHKKVIENVVLSVSIKGAIMVLGFVGYPLVWAAVLADAGTCLLVILNSMILLRDEREAVSTCYRSSTSS
AtHMA2	IILMSNDIRRIPQAIKLAKRAKRKVVENVVISITMKGAILALAFAGHPLIWAAVLADVGTCLLVILNSMLLLSDKHKTGNKCYRESSSS
AtHMA3	PV <mark>KLE</mark> EDEVEDLEV <mark>GLLQK</mark> SE <mark>E</mark>
AtHMA2	VLIAE <mark>KLE</mark> GDAAGDMEAGLLPKISDKHCKPGCCGTKTQEKAMKPAKASSDHSHSGCCETKQKDNVTVV <mark>KKSCC</mark> AEPVDLGHGHDSGCCG
AtHMA2	SGCCS <mark>G</mark> PKDNQQ <mark>K 760 KSQQPHQHEVQVQQSCHNKPSGLD<mark>SGCC</mark>G<mark>G</mark>KSQQPHQHELQQSCHDKPSGLDIGTGPKHEGSSTLVNLEGDAKEELKVLVNGFCSSPAD</mark>
AtHMA2	AITSLKVKSDSHCKSNCSSRERCHHGSNCCRSYAKESCSHDHHHTRAHGVGTLKEIVIE 951 identical similar

## 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.