HvALMT1 from barley is involved in the transport of organic anions

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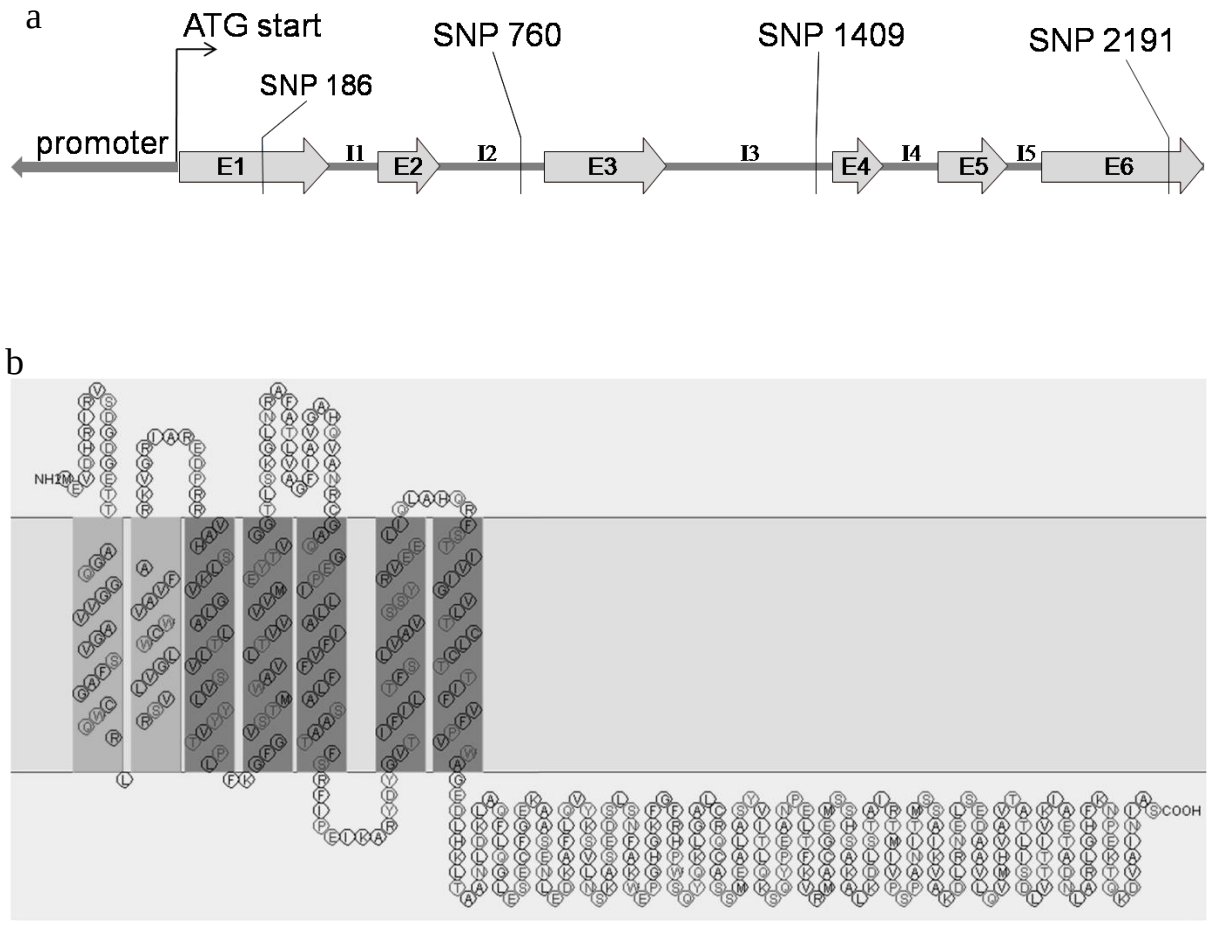
Supplementary Material

Name	Sequence (5'-3')	Description
M13F	GTAAAACGACGGCCAGT	
M13R	AACAGCTATGACCATG	
Hv1-1	GGGTGGCGCACTCGCTC	
Hv1-2	GCTCGACACGGCCACCAG	
Hv1-3	ATGGAGGTTGATCACCGCATC	
Hv1-4	TCAACTCGCAATGTTGATAGCG	
Hv1-5	CGACGTTCTCGCGCTTCAT	
Hv1-6	GACGACGACGACGGTGAGCACG	
Hv1-7	CACCCATGGAGCCAATACCAG	
Hv1-8	GCACTGACGACAAAGAGCTCC	
Hv1-9	GGAATGAATTATCAGAGGACGCAG	
Hv1GFP-1	GCCACTTCAAGAACCCTGAAAAAACTCAGAAAGATGTC GCTATCAACATTGCGAGTATGGTGAGCAAGGGCCACG	*GFP* complementary sequence underlined
Hv1GFP-2	AGCGTGACATAACTAATTACATGATGCGGCCCTCTAGAT GCATGCTCGAG*CCCGGG*TTACTTGTACAGCTCGTCCATG	*Sma*I site italicised, *GFP* complementary sequence underlined
Hv1GFP-3	CTCACTAGA*TTAATTAA*AAGCTTTGAGATTGGCATCG	Forward primer, *PacI* site italicised, overhang underlined
Hv1GFP-4	GATCCAGTG*GGCGCGCC*GGCAGAAATCAGAATGGTGG	Reverse primer, *AscI* site italicised, overhang underlined
Hv1GFP-5	TGCCAACTCACACATACACACACC	Forward promoter sequencing primer
Hv1GFP-6	TTTCCTAGCGTACTTGGGAAGC	Forward promoter sequencing primer
Hv1GFP-7	TTACGTATGATCCATGATCTTCC	Reverse promoter sequencing primer
Hv1GFP-8	CTGTTGGCTGGCTGGTGGCA	Plasmid-localised sequencing primer
Hv1MAP-1	AGGATCGCGAGGGAGGACCCT	
Hv1MAP-2	ATCCGTTTCTAAATATAAGTCTCTTTAA	
Hv1MAP-3	AGGATCGCGAGGGAGGACCCA	

Hv1MAP-4	ATCCGTTTCTAAATATAAGTCTCTTTAG	
Hv2-1	GCTCCGTCAGCGCAATGA	
Hv2-2	TCACTTTGGTTTCAGTTTCTG	
Hv2-3	TCTCGGTGTTCTACTACAC	
Hv2-4	ACTTGTACCCCTCCGCCGC	
Hv2-5	TTCGCGGACCAGGCGGACG	
Hv2-6	CGGTGTTCATGTCGGCCACG	
qRTPCR-1	TTCCTAGCGTCGGCGGCGACG	Forward primer for detection of *HvALMT1*; single underlined text hybridises with exon 2, double underlined text hybridises with exon 3.
qRTPCR-2	ATGAGCTCCTCCACGCGGTAGC	Reverse primer for detection of *HvALMT1*
qRTPCR-3	GTGAGGCTGGTGCTGATTACG	Forward primer for detection of *HvGAPDH*; Burton *et al*., (2004).
qRTPCR-4	TGGTGCAGCTAGCATTTGAGAC	Reverse primer for detection of *HvGAPDH*; Burton *et al*., (2004).
TaALMT1F	GGAATGGAATTCAACTGCTTTGGCG	Forward primer for detection of *TaALMT1*
TaALMT1R	TCCTCAGTGGCCTTCGAATTAAGG	Reverse primer for detection of *TaALMT1*

Supplementary Table 1. Primers used for PCR.

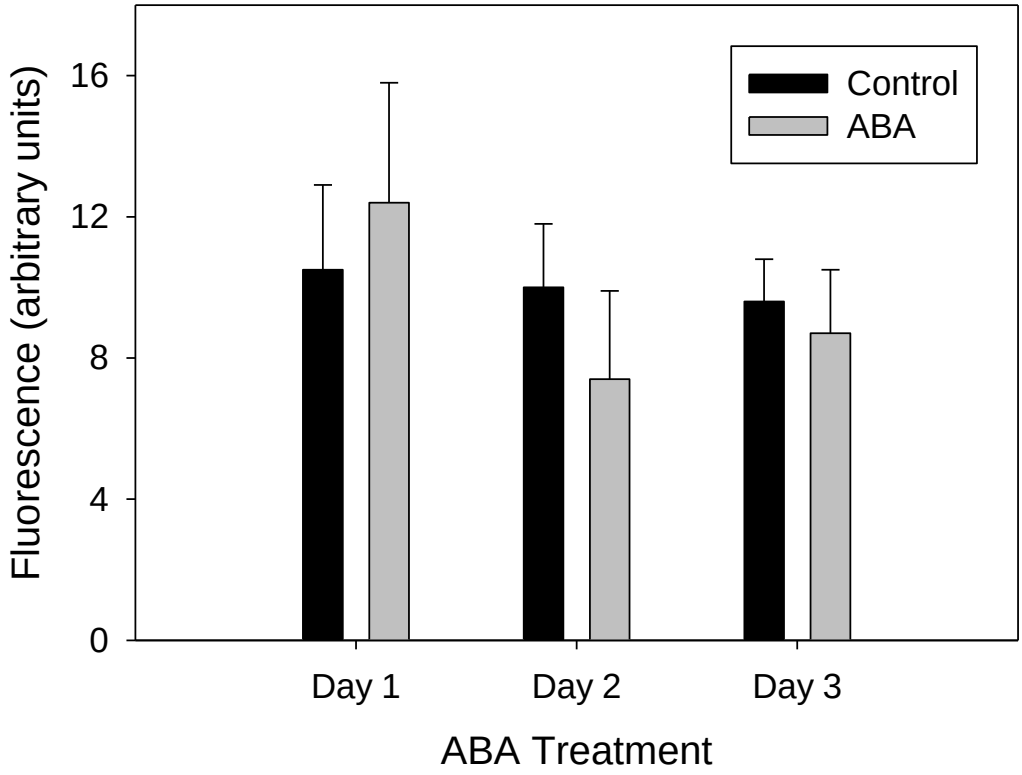


Supplementary Figure 1. The genomic structure and hydropathy plot of HvALMT1. The genomic structure of *HvALMT1* is shown in panel a indicating the position of the ATG start site, exons one to six (E1 to E6) and introns one to five (I1 to I5). The position of the single nucleotide polymorphisms (SNP's) are indicated along with the nucleotide position from the ATG start site. The promoter region is indicated upstream of the ATG start site. The transmembrane topology of the HvALMT1 protein as predicted by SOSUI v.1.11 is indicated in panel b.

Dayton	[184] CC**T**CG	[758] CT**T**TA	[1407]GAGTT	[2189]CTGCT
Morex	CCACG	CTCTA	GAGTT	CT**A**CT
Golden Promise	CCACG	CTCTA	GA**A**TT	CTGCT
Zhepi 2	CCACG	CTCTA	GA**A**TT	CTGCT

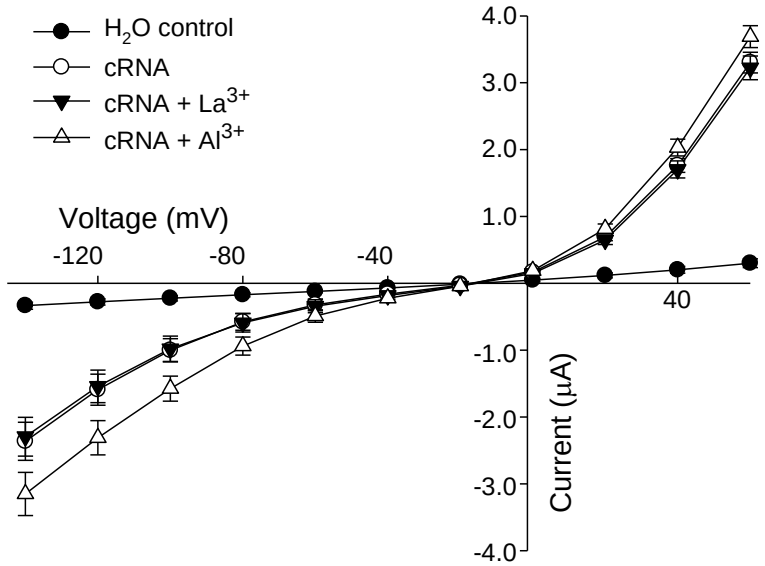
Supplementary Figure 2. Single nucleotide polymorphisms (SNPs) in *HvALMT1* between four barley cultivars.

Partial nucleotide sequences detailing the SNPs (in bold) are shown for each of the four cultivars. The last SNP results in a change of amino acid at position 431 from an alanine in cultivars Dayton, Golden Promise and Zhepi 2 to a threonine in cultivar Morex. The numbers indicate the base number of the first nucleotide shown in each set counted from the ATG start site of *HvALMT1*.



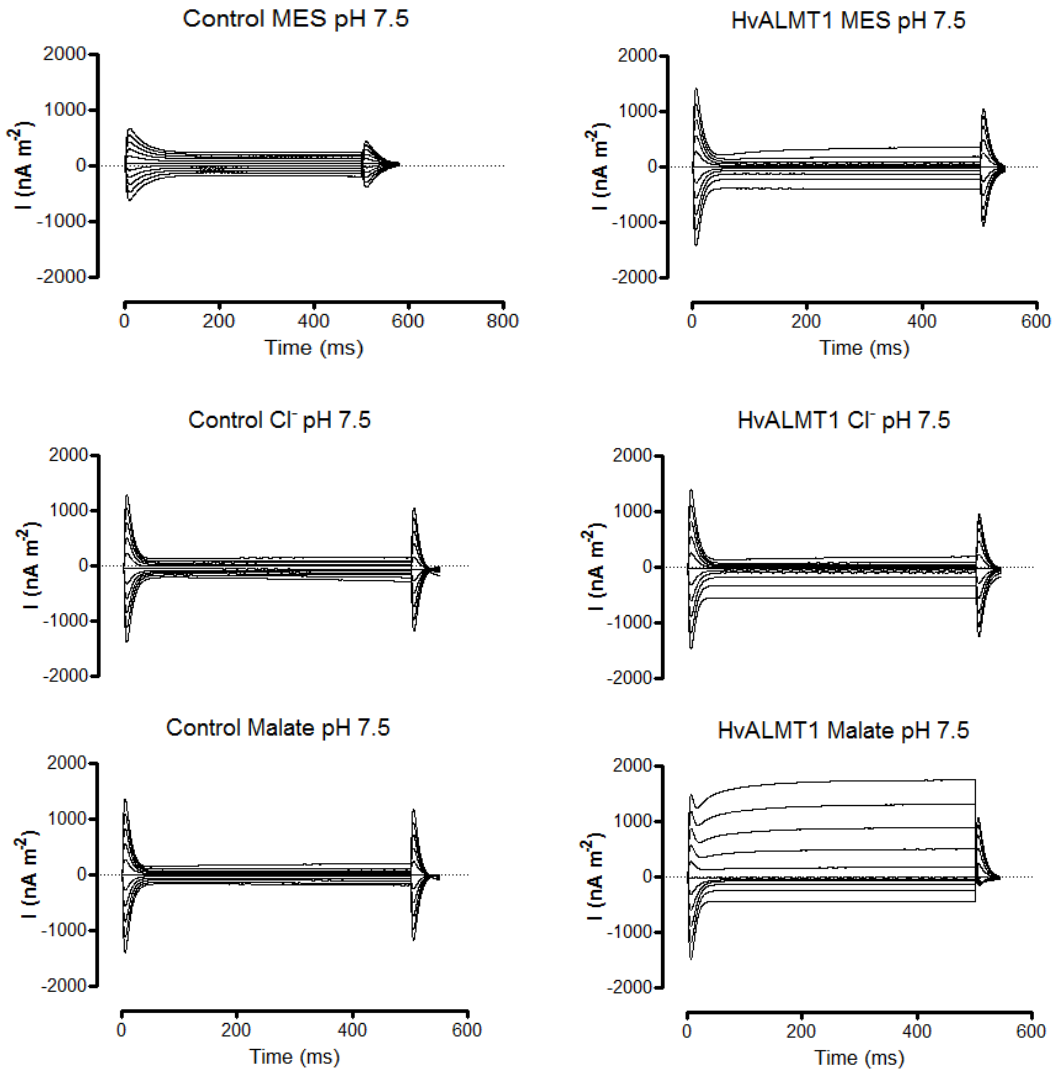
Supplementary Figure 3. Effect of ABA treatment on fluorescence of guard cells expressing GFP driven by the *HvALMT1* promoter.

Seedlings were sprayed with a solution that contained 1mM ABA (ABA) or with the same solution that lacked ABA (Control) and analysed for GFP fluorescence in guard cells over 3 days. Data are expressed as arbitrary units and represent the mean and standard error of four or five leaves. For each leaf five guard cells were analysed and the mean of these were used to calculate a grand mean.



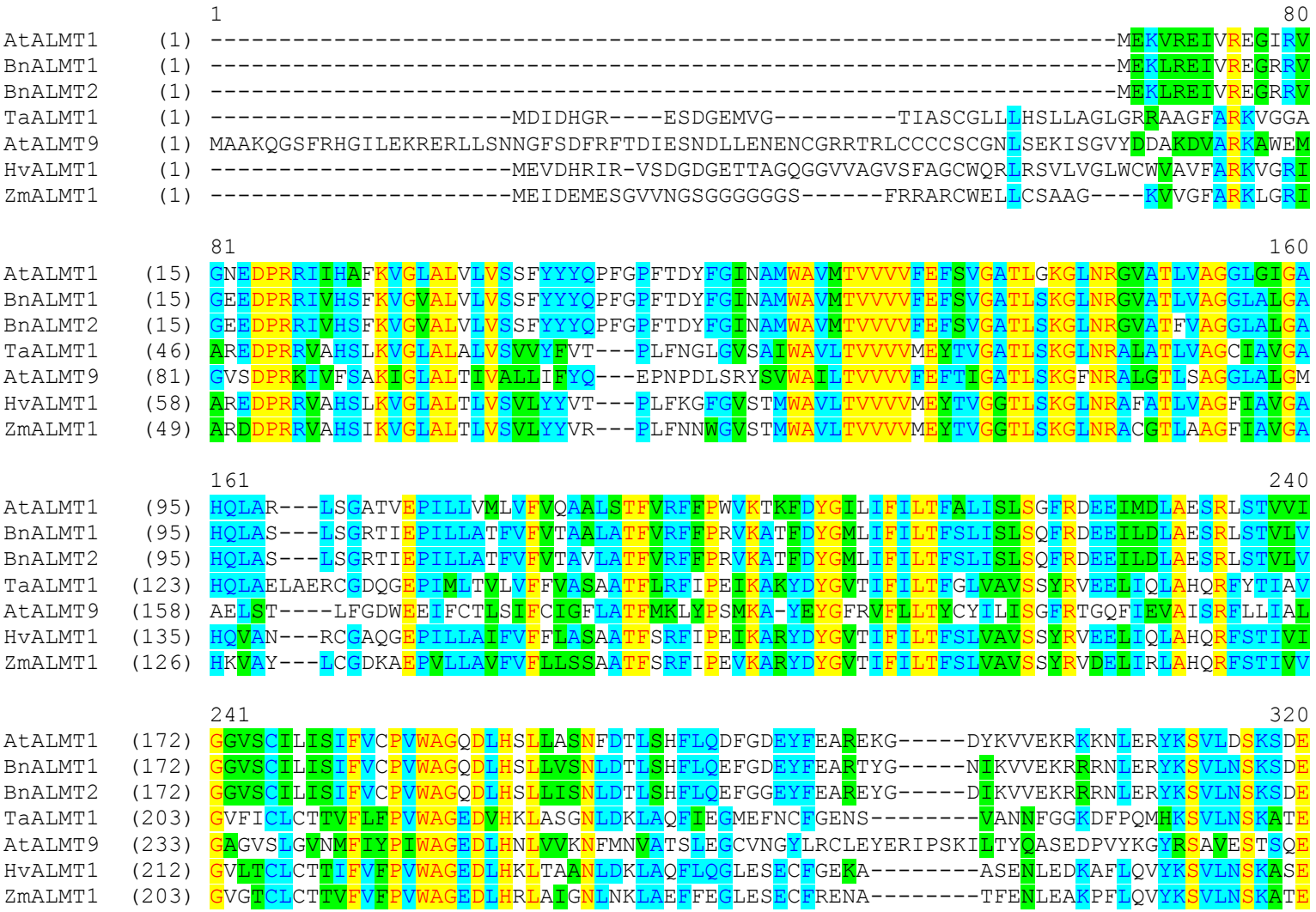
Supplementary Figure 4. Effect of Al^{3+} on currents in *Xenopus* oocytes expressing *HvALMT1*.

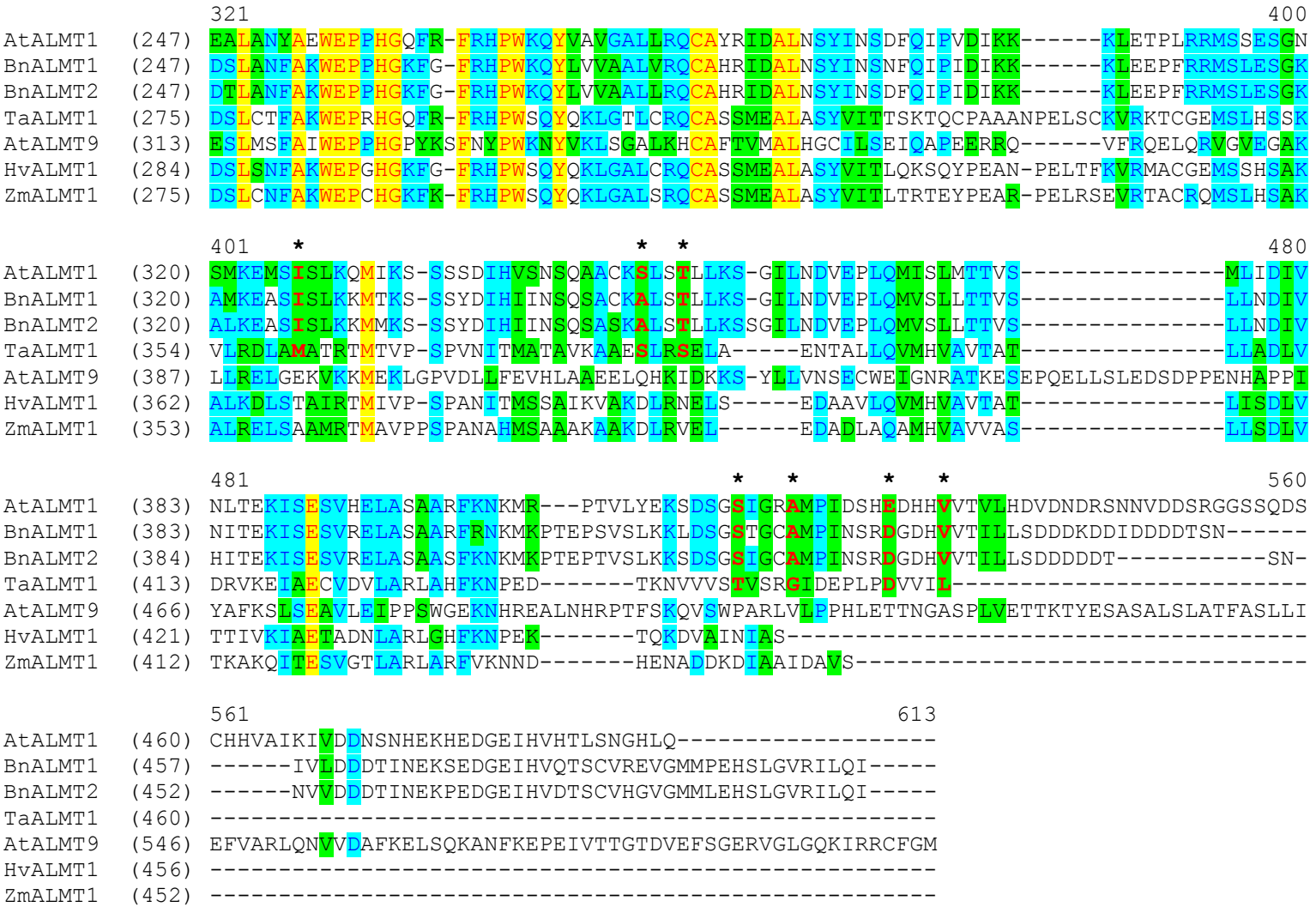
Xenopus laevis oocytes were injected with either water as a control (H_2O control) or with *HvALMT1* cRNA (cRNA). After 2 d oocytes were subsequently injected with malate and currents recorded from oocytes incubated in a buffer at pH 4.5 with no added trivalent cations (cRNA), in the presence of 100 μM $LaCl_3$ (cRNA + La^{3+}) or in the presence of 100 μM $AlCl_3$ (cRNA + Al^{3+}). H_2O controls treated with 100 μM of either $LaCl_3$ or $AlCl_3$ generated similar curves to the H_2O control shown above and are not included in the figure. Error bars show SEM, n = 7 (cRNA) and n = 5 (H_2O control).



Supplementary Figure 5 *HvALMT1*-dependent currents measured in oocytes at pH 7.5.

Family of current curves measured in *Xenopus laevis* oocytes injected with water (Control) or *HvALMT1* cRNA in response to 500 ms voltage pulses between -140 mV to 60 mV in 20 mV steps. The data were collected with either 10 mM MES-BTP, 10 mM Cl^- -BTP or 10 mM malate-BTP in the bathing solution (pH 7.5


1 80
AtALMT1 (1) ----------MEKVREIVREGIRV
BnALMT1 (1) ----------MEKLREIVREGRRV
BnALMT2 (1) ----------MEKLREIVREGRRV
TaALMT1 (1) ----------MDIDHGR----ESDGEMVG---------TIASCGLLLHSLLAGLGRRAAGFARKVGGA
AtALMT9 (1) MAAKQGSFRHGILEKRERLLSNNGFSDFRFTDIESNDLLENENCGRRTRLCCCCSCGNLSEKISGVYDDAKDVARKAWEM
HvALMT1 (1) ----------MEVDHRIR-VSDGDGETTAGQGGVVAGVSFAGCWQRLRSVLVGLWCWVAVFARKVGRI
ZmALMT1 (1) ----------MEIDEMESGVVNGSGGGGGGS------FRRARCWELLCSAAG----KVVGFARKLGRI
81 160
AtALMT1 (15) GNEDPRRIIHAFKVGLALVLVSSFYYYQPFGPFTDYFGINAMWAVMTVVVVFEFSVGATLGKGLNRGVATLVAGGLGIGA
BnALMT1 (15) GEEDPRRIVHSFKVGVALVLVSSFYYYQPFGPFTDYFGINAMWAVMTVVVVFEFSVGATLSKGLNRGVATLVAGGLALGA
BnALMT2 (15) GEEDPRRIVHSFKVGVALVLVSSFYYYQPFGPFTDYFGINAMWAVMTVVVVFEFSVGATLSKGLNRGVATFVAGGLALGA
TaALMT1 (46) AREDPRRVAHSLKVGLALALVSVVYFVT---PLFNGLGVSAIWAVLTVVVVMEYTVGATLSKGLNRALATLVAGCIAVGA
AtALMT9 (81) GVSDPRKIVFSAKIGLALTIVALLIFYQ---EPNPDLSRYSVWAILTVVVVFEFTIGATLSKGFNRALGTLSAGGLALGM
HvALMT1 (58) AREDPRRVAHSLKVGLALTLVSVLYYVT---PLFKGFGVSTMWAVLTVVVVMEYTVGGTLSKGLNRAFATLVAGFIAVGA
ZmALMT1 (49) ARDDPRRVAHSIKVGLALTLVSVLYYVR---PLFNNWGVSTMWAVLTVVVVMEYTVGGTLSKGLNRACGTLAAGFIAVGA
161 240
AtALMT1 (95) HQLAR---LSGATVEPILLVMLVFVQAALSTFVRFFPWVKTKFDYGILIFILTFALISLSGFRDEEIMDLAESRLSTVVI
BnALMT1 (95) HQLAS---LSGRTIEPILLATFVFVTAALATFVRFFPRVKATFDYGMLIFILTFSLISLSQFRDEEILDLAESRLSTVLV
BnALMT2 (95) HQLAS---LSGRTIEPILLATFVFVTAVLATFVRFFPRVKATFDYGMLIFILTFSLISLSQFRDEEILDLAESRLSTVLV
TaALMT1 (123) HQLAELAERCGDQGEPIMLTVLVFFVASAATFLRFIPEIKAKYDYGVTIFILTFGLVAVSSYRVEELIQLAHQRFYTIAV
AtALMT9 (158) AELST----LFGDWEEIFCTLSIFCIGFLATFMKLYPSMKA-YEYGFRVFLLTYCYILISGFRTGQFIEVAISRFLLIAL
HvALMT1 (135) HQVAN---RCGAQGEPILLAIFVFFLASAATFSRFIPEIKARYDYGVTIFILTFSLVAVSSYRVEELIQLAHQRFSTIVI
ZmALMT1 (126) HKVAY---LCGDKAEPVLLAVFVFLLSSAATFSRFIPEVKARYDYGVTIFILTFSLVAVSSYRVDELIRLAHQRFSTIVV
241 320
AtALMT1 (172) GGVSCILISIFVCPVWAGQDLHSLLASNFDTLSHFLQDFGDEYFEAREKG-----DYKVVEKRKKNLERYKSVLDSKSDE
BnALMT1 (172) GGVSCILISIFVCPVWAGQDLHSLLVSNLDTLSHFLQEFGDEYFEARTYG-----NIKVVEKRRRNLERYKSVLNSKSDE
BnALMT2 (172) GGVSCILISIFVCPVWAGQDLHSLLISNLDTLSHFLQEFGGEYFEAREYG-----DIKVVEKRRRNLERYKSVLNSKSDE
TaALMT1 (203) GVFICLCTTVFLFPVWAGEDVHKLASGNLDKLAQFIEGMEFNCFGENS--------VANNFGGKDFPQMHKSVLNSKATE
AtALMT9 (233) GAGVSLGVNMFIYPIWAGEDLHNLVVKNFMNVATSLEGCVNGYLRCLEYERIPSKILTYQASEDPVYKGYRSAVESTSQE
HvALMT1 (212) GVLTCLCTTIFVFPVWAGEDLHKLTAANLDKLAQFLQGLESECFGEKA--------ASENLEDKAFLQVYKSVLNSKASE
ZmALMT1 (203) GVGTCLCTTVFVFPVWAGEDLHRLAIGNLNKLAEFFEGLESECFRENA--------TFENLEAKPFLQVYKSVLNSKATE


321 400
AtALMT1 (247) EALANYAEWEPPHGQFR-FRHPWKQYVAVGALLRQCAYRIDALNSYINSDFQIPVDIKK------KLETPLRRMSSESGN
BnALMT1 (247) DSLANFAKWEPPHGKFG-FRHPWKQYLVVAALVRQCAHRIDALNSYINSNFQIPIDIKK------KLEEPFRRMSLESGK
BnALMT2 (247) DTLANFAKWEPPHGKFG-FRHPWKQYLVVAALLRQCAHRIDALNSYINSDFQIPIDIKK------KLEEPFRRMSLESGK
TaALMT1 (275) DSLCTFAKWEPRHGQFR-FRHPWSQYQKLGTLCRQCASSMEALASYVITTSKTQCPAAANPELSCKVRKTCGEMSLHSSK
AtALMT9 (313) ESLMSFAIWEPPHGPYKSFNYPWKNYVKLSGALKHCAFTVMALHGCILSEIQAPEERRQ------VFRQELQRVGVEGAK
HvALMT1 (284) DSLSNFAKWEPGHGKFG-FRHPWSQYQKLGALCRQCASSMEALASYVITLQKSQYPEAN-PELTFKVRMACGEMSSHSAK
ZmALMT1 (275) DSLCNFAKWEPCHGKFK-FRHPWSQYQKLGALSRQCASSMEALASYVITLTRTEYPEAR-PELRSEVRTACRQMSLHSAK
401 480
AtALMT1 (320) SMKEMSISLKQMIKS-SSSDIHVSNSQAACKSLSTLLKS-GILNDVEPLQMISLMTTVS---------------MLIDIV
BnALMT1 (320) AMKEASISLKKMTKS-SSYDIHIINSQSACKALSTLLKS-GILNDVEPLQMVSLLTTVS---------------LLNDIV
BnALMT2 (320) ALKEASISLKKMMKS-SSYDIHIINSQSASKALSTLLKSSGILNDVEPLQMVSLLTTVS---------------LLNDIV
TaALMT1 (354) VLRDLAMATRTMTVP-SPVNITMATAVKAAESLRSELA-----ENTALLQVMHVAVTAT---------------LLADLV
AtALMT9 (387) LLRELGEKVKKMEKLGPVDLLFEVHLAAEELQHKIDKKS-YLLVNSECWEIGNRATKESEPQELLSLEDSDPPENHAPPI
HvALMT1 (362) ALKDLSTAIRTMIVP-SPANITMSSAIKVAKDLRNELS-----EDAAVLQVMHVAVTAT---------------LISDLV
ZmALMT1 (353) ALRELSAAMRTMAVPPSPANAHMSAAAKAAKDLRVEL------EDADLAQAMHVAVVAS---------------LLSDLV
481 560
AtALMT1 (383) NLTEKISESVHELASAARFKNKMR---PTVLYEKSDSGSIGRAMPIDSHEDHHVVTVLHDVDNDRSNNVDDSRGGSSQDS
BnALMT1 (383) NITEKISESVRELASAARFRNKMKPTEPSVSLKKLDSGSTGCAMPINSRDGDHVVTILLSDDDKDDIDDDDTSN------
BnALMT2 (384) HITEKISESVRELASAASFKNKMKPTEPTVSLKKSDSGSIGCAMPINSRDGDHVVTILLSDDDDDT-----------SN-
TaALMT1 (413) DRVKEIAECVDVLARLAHFKNPED-------TKNVVVSTVSRGIDEPLPDVVIL--------------------------
AtALMT9 (466) YAFKSLSEAVLEIPPSWGEKNHREALNHRPTFSKQVSWPARLVLPPHLETTNGASPLVETTKTYESASALSLATFASLLI
HvALMT1 (421) TTIVKIAETADNLARLGHFKNPEK-------TQKDVAINIAS-----------------------------------
ZmALMT1 (412) TKAKQITESVGTLARLARFVKNND-------HENADDKDIAAIDAVS-----------------------------------
561 613
AtALMT1 (460) CHHVAIKIVDDNSNHEKHEDGEIHVHTLSNGHLQ-------------------
BnALMT1 (457) ------IVLDDDTINEKSEDGEIHVQTSCVREVGMMPEHSLGVRILQI-----
BnALMT2 (452) ------NVVDDDTINEKPEDGEIHVDTSCVHGVGMMLEHSLGVRILQI-----
TaALMT1 (460) --
AtALMT9 (546) EFVARLQNVVDAFKELSQKANFKEPEIVTTGTDVEFSGERVGLGQKIRRCFGM
HvALMT1 (456) --
ZmALMT1 (452) --

Supplementary Figure 6. Alignments of functionally characterised ALMT proteins.

All seven proteins have been expressed and characterised for transport activity in *Xenopus* oocytes. The top four proteins in the alignment (AtALMT1, BnALMT1, BnALMT2 and TaALMT1) are all activated by Al^{3+} (Ligaba *et al.*, 2006, Sasaki *et al.*, 2004, Hoekenga *et al.*, 2006) whereas the bottom three proteins (AtALMT9, HvALMT1 and ZmALMT1) show little or no response to Al^{3+} treatment (Kovermann *et al.*, 2007, Pineros *et al*. 2008b, Supplementary Figure S3). However, for AtALMT9, Al^{3+} was added to a solution of pH 7.5 which would effectively result in little or no Al^{3+} in solution (Kovermann *et al.*, 2007) and was included since its membrane location (tonoplast) would suggest that the protein is not likely to be Al^{3+} activated. The sequences were aligned using the Clustal W algorithm in the VectorNTI software package (version 11). Identical amino acid residues shared by all proteins have a yellow background, identical residues shared by four or more sequences have a blue background and strongly similar residues shared by four or more sequences have a green background. The asterisks and red letters denote strongly similar amino acid residues present in all four Al^{3+} activated proteins and absent from the other proteins. Accession numbers (in bold) for the various proteins are AtALMT1: **AAF22890**; AtALMT9: **NP_188473**; BnALMT1: **BAE97280**; BnALMT2: **BAE97281**; ZmALMT1: **ABC86748**; TaALMT1: **BAD10882** and HvALMT1: **EF424084**.

GFP Movies 1 and 2. Time-series videos of fluorescence from the HvALMT1:GFP fusion.

Shown are two time-series videos of fluorescence from cells that are transiently expressing the HvALMT1:GFP reporter fusion. Movie 1 shows a cell where the nucleus is prominent and movie 2 a cell where a GFP signal is also present at the plasma membrane. Images were acquired approximately every 5.8 seconds.