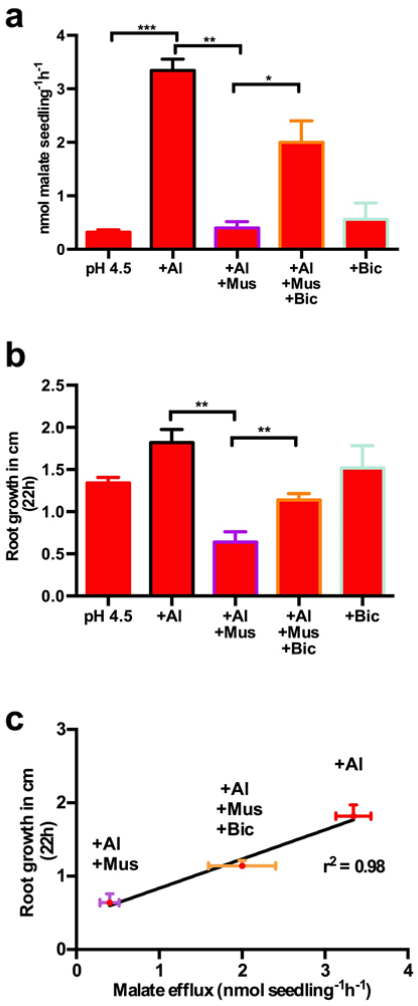
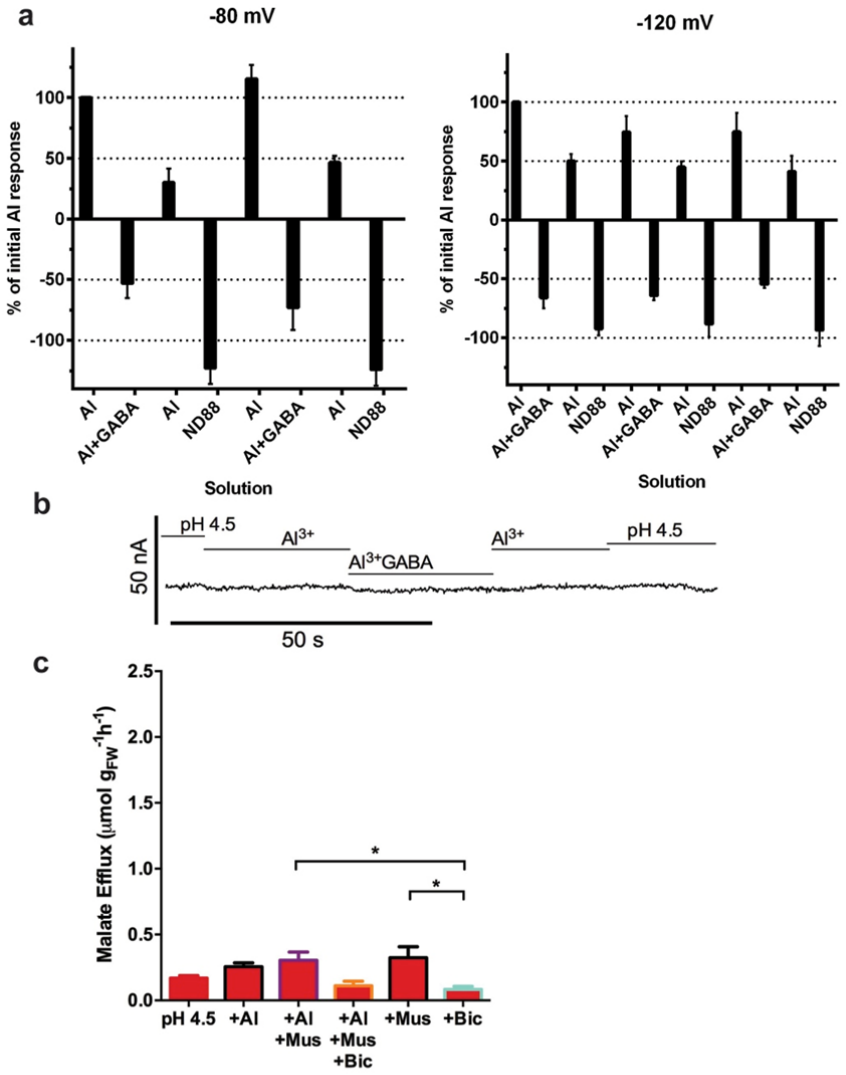
Supplementary Data

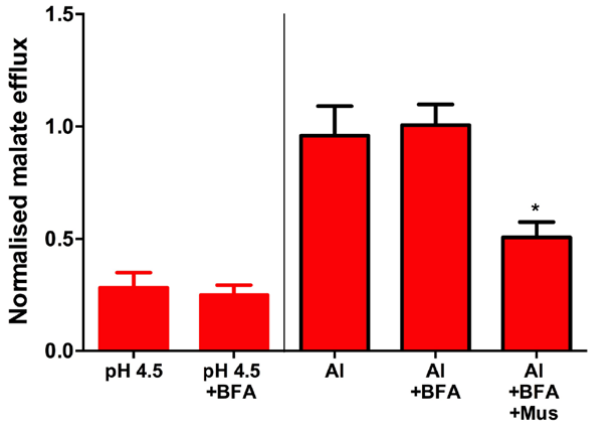
Supplementary Figures



Supplementary Figure 1 Bicuculline abolishes muscimol regulation of Al^{3+} tolerance of ET8 wheat roots[8]. Muscimol (Mus, 10 μM) in the presence of 100 μM $AlCl_3$ (Al) at pH 4.5 inhibited **a**, malate efflux and **b**, growth, whereas 100 μM bicuculline (Bic) abolished the effect of muscimol. **c**, linear regression showing strong significant correlation between malate flux and growth in presence of combinations of Al, Mus and Bic. All data n = 5, *, ** and *** indicates significance at 0.05, 0.01 and 0.001 respectively of relevant comparisons using a two-tailed t-test.

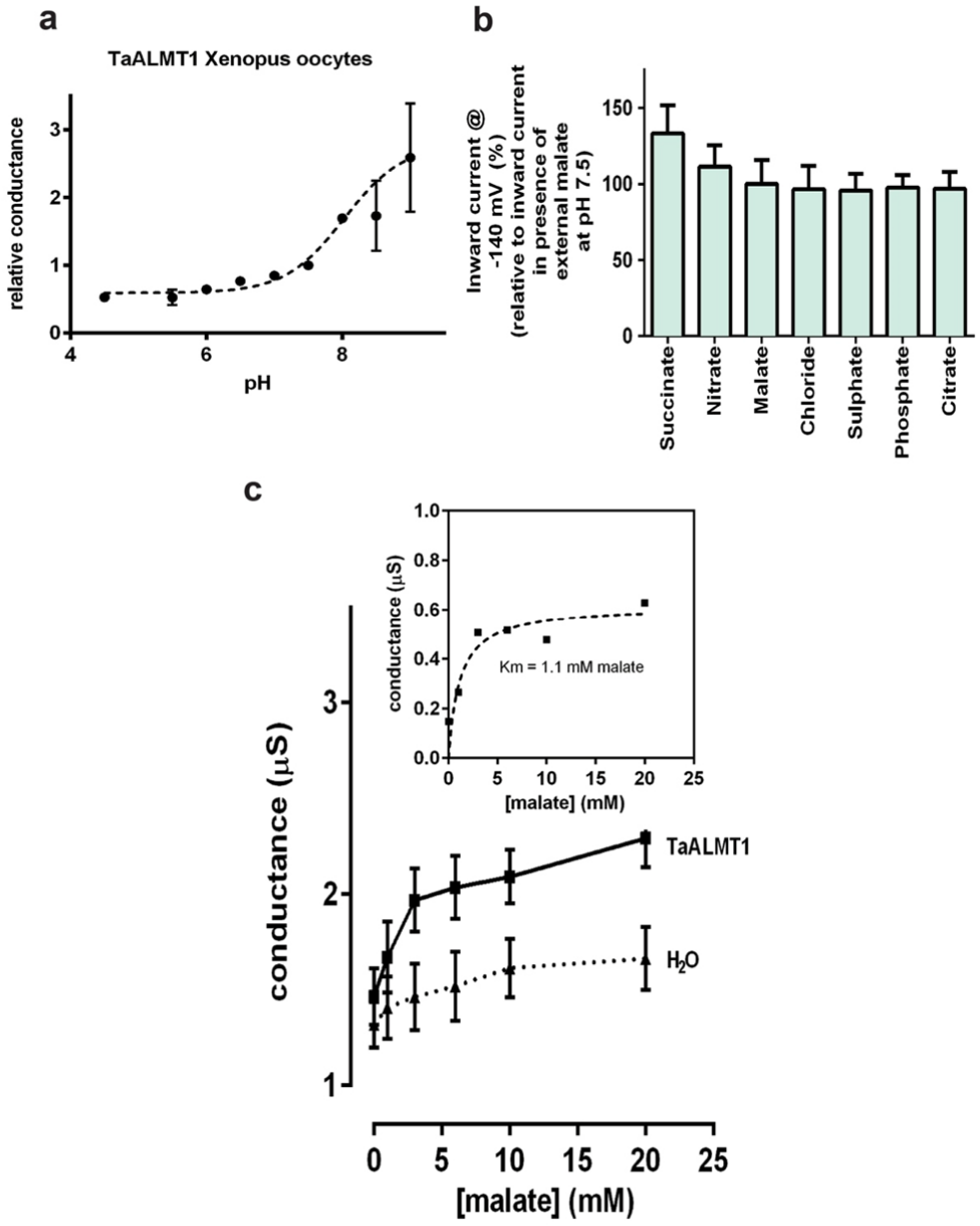


Supplementary Figure 2 GABA and muscimol regulate Al^{3+}-activated malate currents and fluxes through TaALMT1. a, Percentage activation of malate efflux by Al^{3+} and inhibition of this current by 100 µM GABA in *TaALMT1*-injected *X. laevis* oocytes. Sequential solution changes (30 s in each solution) were made over 6 min at -80 mV (left) and -120 mV (right). These experiments demonstrate reversibility and lack of rundown. n = 8 independent oocytes. **b**, Representative current traces at -120 mV of water-injected control *X. laevis* oocytes bathed in ND88 at pH 4.5 ± 100 µM Al^{3+} ± 100 µM GABA, control for Fig. 2b. **c**, Malate fluxes from vector control transformed BY2 cells, control for Fig. 2c, n=5. Scaling equivalent to Fig. 2c.

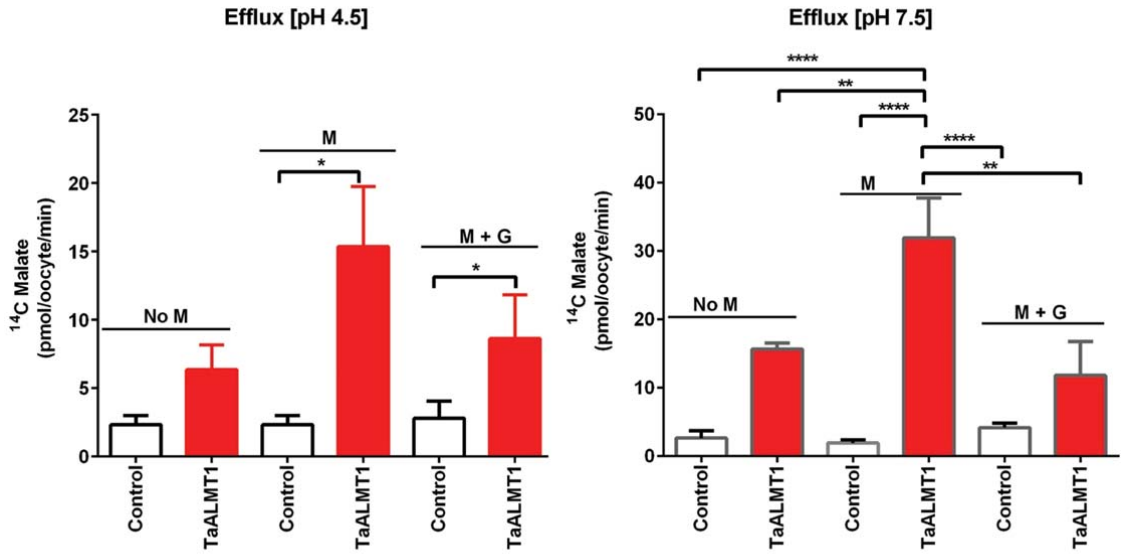


Supplementary Figure 3 The endocytosis inhibitor Brefeldin A (BFA) had no effect on the Al^{3+}-activated malate efflux carried by TaALMT1 or its muscimol inhibition.

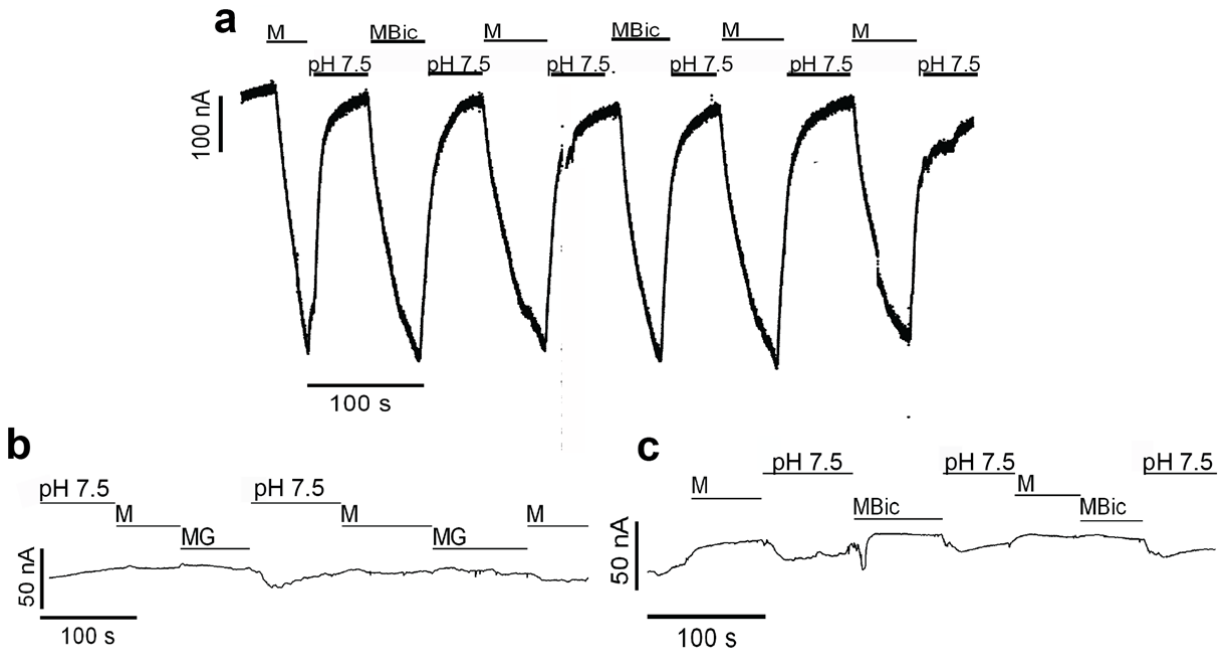
Tobacco BY2 cells expressing *TaALMT1* were placed in 3 mM $CaCl_2$ + 5 mM MES, pH 4.5 overnight for 20 h followed by transfer to same solution but ± BFA (100 μM) or $AlCl_3$ (100 μM) ± BFA ± Musc (10 μM) for 1 h. Solutions were buffered with BTP. Supernatant was harvested and assayed for malate fluxes. All fluxes were normalized to value for +Al.



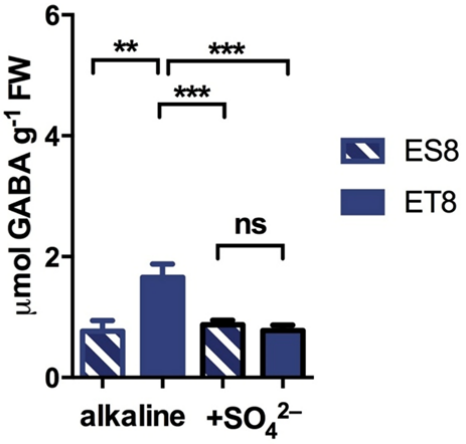
Supplementary Figure 4 External anions activate TaALMT1 in *X. laevis* oocytes. **a,** Relative slope conductance of TaALMT1-injected oocytes, normalised to value at pH 7.5 (in the absence of Al^{3+}), n = 5. **b**, No significant difference between activation of TaALMT1 by 10 mM of each anion applied externally using a one-way ANOVA. **c,** Slope conductance (–100 to –140 mV) vs current of *TaALMT1*- or water-injected *X. laevis* oocytes. Michaelis-Menten fit of control subtracted TaALMT1-mediated currents (inset). All oocytes were pre-injected to contain ~10 mM malate.



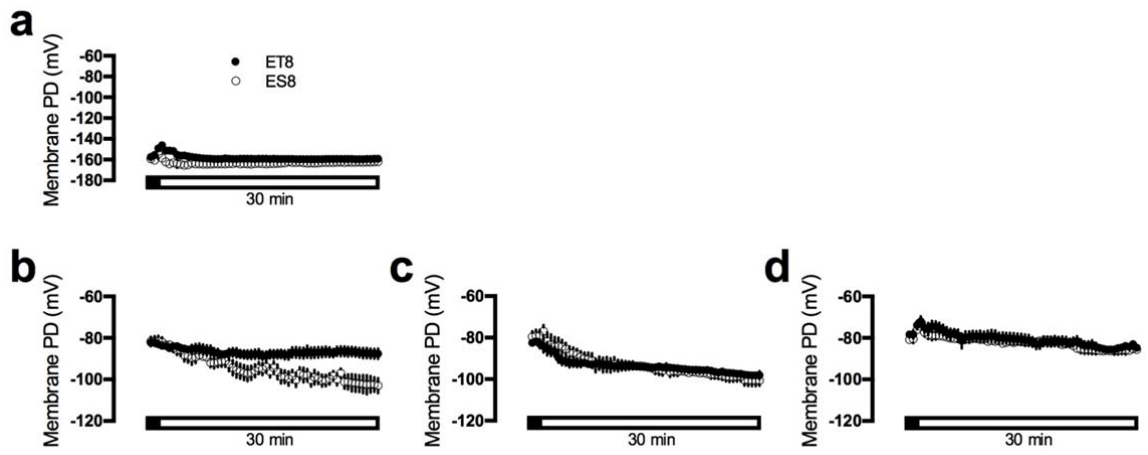
Supplementary Figure 5 Anion activation of TaALMT1 is greater at alkaline pH and modulated by GABA. *X. laevis* oocytes injected with TaALMT1 cRNA or water were preloaded with [^{14}C] malate and exposed to solution containing malate (M, 10 mM) ± GABA (G, 100 µM). Malate efflux was measured at pH 7.5 or 4.5. All data is mean efflux of radioactive malate from the oocytes 2 min after injection (n = 5). *, **, and **** indicates significant differences between genotypes at $p<0.05$, 0.01, and 0.0001 respectively using a one way ANOVA and tukey's post-hoc test.



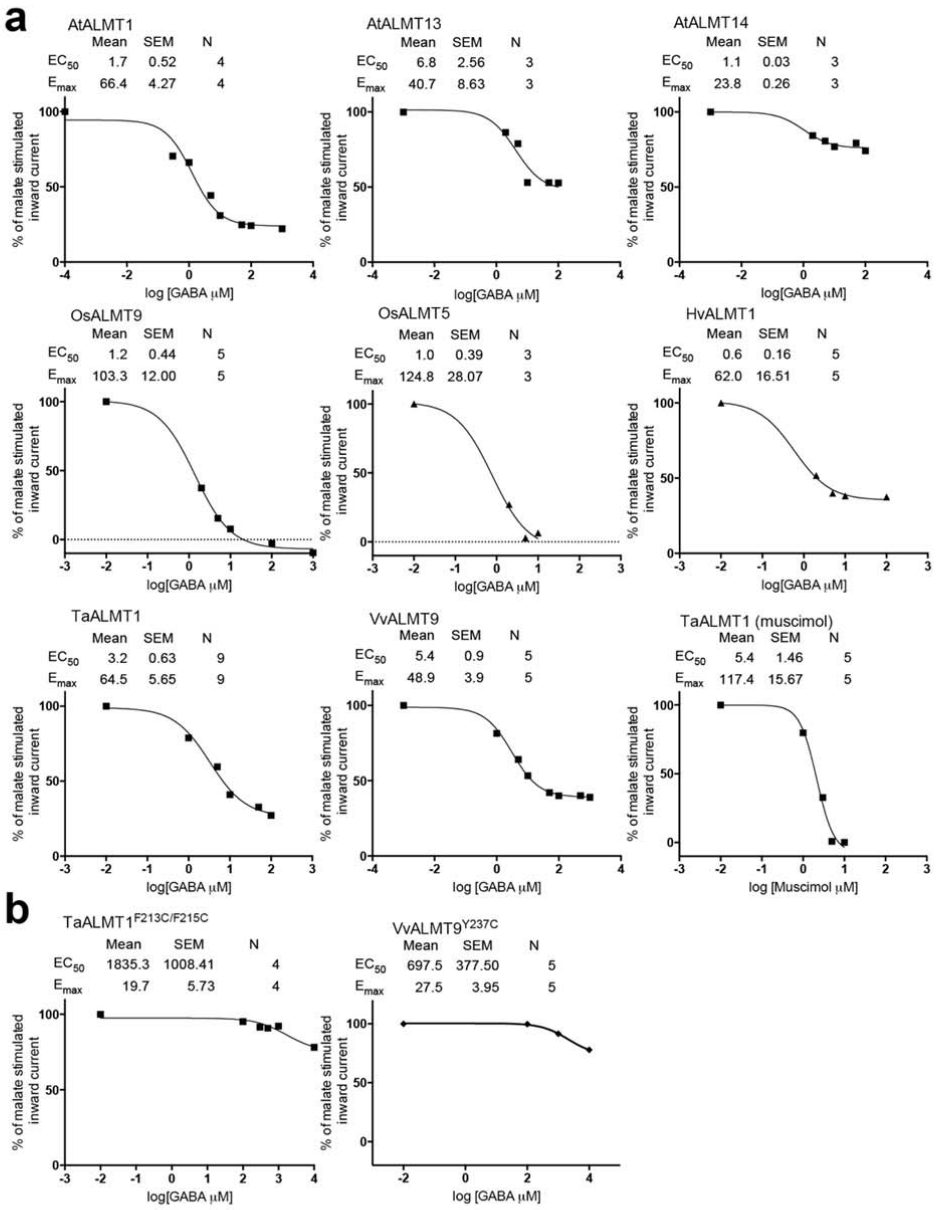
Supplementary Figure 6 GABA negatively regulates anion-activated malate currents through TaALMT1. In *TaALMT1*-injected *X. laevis* oocytes **r**epresentative current traces at -120 mV showing **a,** 100 µM bicuculline (Bic) has no effect on malate activated currents in the absence of GABA at pH 7.5. **b**,**c**, In water injected control oocytes, malate, bicuculline and GABA have no effect on currents at -120 mV. These traces are the controls for Fig. 4a.



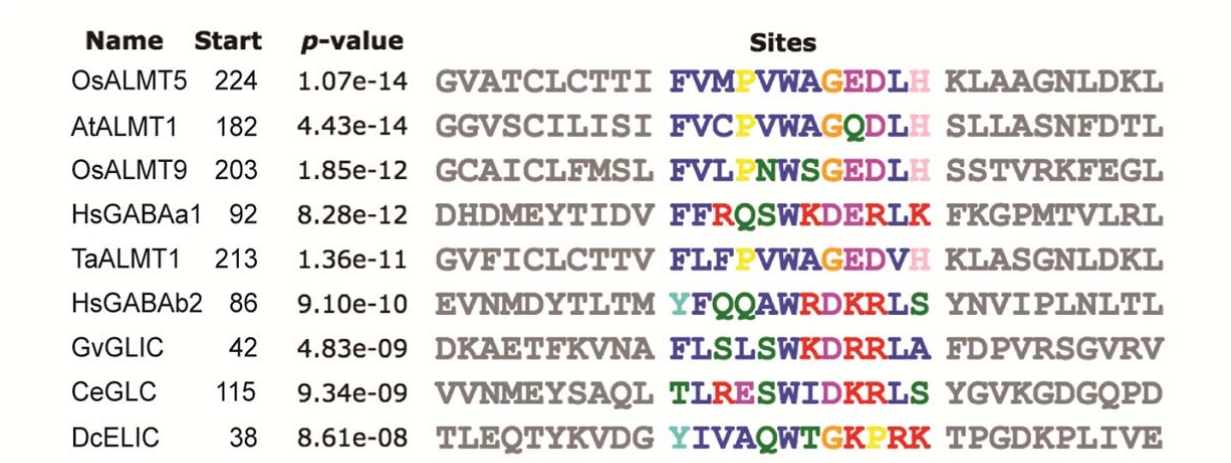
Supplementary Figure 7 Effect of alkaline pH on GABA concentration in wheat NIL ET8 and ES8[8]. GABA concentration of wheat roots bathed in basal solution at pH 7.5 ± 10 mM SO_4^{2-}. n = 11 from 3 individual experiments. ***, and **** indicates significant differences between genotypes at p< 0.001 and 0.0001 respectively using a one way ANOVA and tukey's post-hoc test, ns, not significant. Scaling equivalent to Fig. 1a.



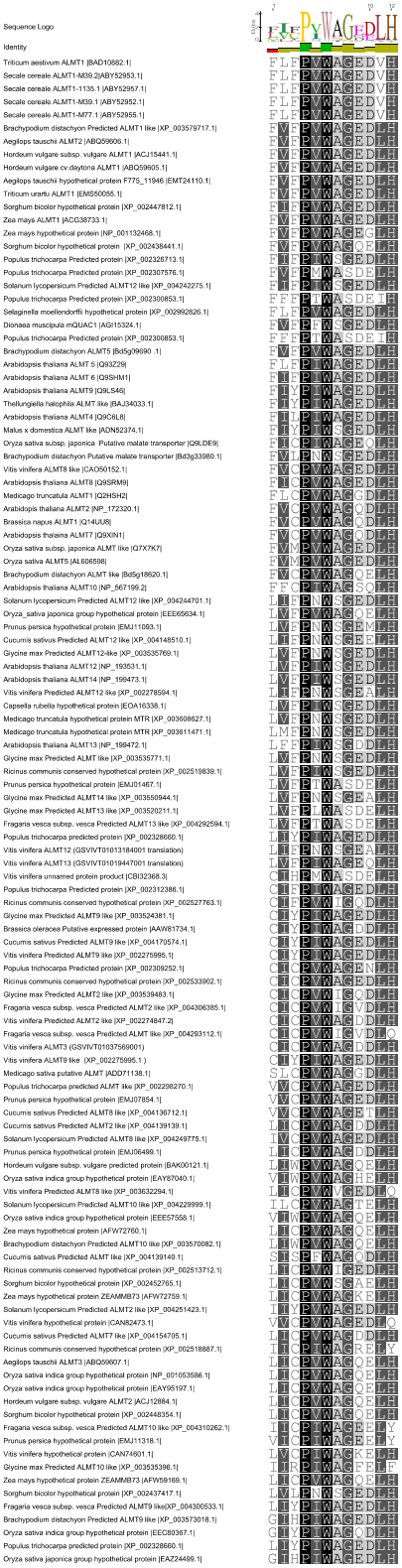
Supplementary Figure 8 Membrane potential difference (PD) across plasma membrane of wheat NIL ET8 and ES8[8] root apical cells in response to muscimol, and to Al^{3+}. a, Control for Fig. 5c,d; no differential response between genotypes when exposed to 10 µM muscimol in the absence of a transactivating anion, 10 mM SO_4^{2-} at pH 8, n = 3 per genotype. **b**, Membrane potential after 100 µM Al^{3+} treatment addition at pH 4.5. **c,** membrane potential after simultaneous addition of 100 µM Al^{3+} and 10 µM Mus treatment at pH 4.5, n = 7 per genotype. **d**, Membrane potential after 10 µM Mus treatment at pH 4.5, n = 6 per genotype. Black scale bar indicates value prior to treatment, clear bar is in presence of treatment.



Supplementary Figure 9. The affinity of GABA (and muscimol) regulation of anion-activated currents through various plant ALMT expressed in *X. laevis* oocytes. a, wildtype proteins **b,** mutagenised TaALMT$^{F213C/F215C}$ or VvALMT9^{Y237C}. All experiments performed in basal solutions + symmetrical 10 mM malate with current at –140 mV taken 30 s after each solution change. Data is control subtracted, presented is example of one oocyte for each gene, and the table shows statistics for repeat experiments



Supplementary Figure 10 Variation in the consensus motif identified between ALMT and bacterial and animal ion channels. MEME output[18] for selected ALMT and ion channels used in constructing the human $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor model[16] which derived the motif of interest [FY][LVF]R[PQ][SV]W[AK][GD][EK][DR]L[HKS]. For full sequences see NCBI/GenBank/EMBL databases under accession numbers HsGABAa1 (NP_507090.2); HsGABAb2 (NP_000804.1); CeGLC1 (NP_507090.1); GvGLIC (568786838); DcELIC (P0C7B7.1).



Supplementary Figure 11 Sequence alignment of 116 ALMT using consensus motif as a BLAST search string. Highly conserved (100%) residues are shaded in black, 80% similar are shaded grey, while <60% similar are unshaded. Performed using CLUSTAL W2 and Geneious (version 6.1.5).

Polarity/Charge		Amino acids	1	2	3	4	5	6	7	8	9	10	11	12
Hydrophobic		Alanine [A]	0	0	0	0	0	0	75.7	0	0.9	2.6	0	0
Hydrophilic		Cysteine [C]	12.9	0	35.3	0	0	0	0	0	0	0	0	0
Negatively charged		Aspartic acid [D]	0	0	0	0	0	0	0	0	12.2	65.2	0	0
Negatively charged		Glutamic acid [E]	0	0	0	0	0	0	0	0	60.0	26.1	0	0
Hydrophobic		Phenylalanine [F]	36.2	3.5	40.5	0	1.7	0	0	0	0.9		0	0.9
Hydrophobic		Glycine [G]	2.6	0	0	0	0	0	0	93.9	1.7	0.9	0	0
Positively charged		Histidine [H]	0	0	2.6	0	0	0	0	0	0.9	0	0	93.9
Hydrophobic		Isoleucine [Ile]	5.2	51.3	0	0	28.7	0	4.3	0	0.0	0	1.7	0
Positively charged		Lysine [K]	0	0	0	0	0	0	0	0	1.7	0	0	0
Hydrophobic		Leucine [L]	35.3	8.7	2.6	0	0.9	0	0	0	0	0	94.0	0
Hydrophobic		Methionine [M]	0	0.9	1.7	0	1.7	0	0	0	0	0.9	0	0
Hydrophilic		Aspargine [N]	0	0	0	0	10.4	0	0	0	0	0.9	0	0
Hydrophobic		Proline [P]	0	0	0	100.0	0	0	0	0	0	0	0	0
Hydrophilic		Glutamine [Q]	0	0	0	0	0	0	0	0	17.4	2.6	0	2.6
Positively charged		Arginine [R]	0	0	0.9	0	0	0	0	0	0.9	0	0	0
Hydrophilic		Serine [S]	1.7	0	0.9	0	0	0	19.1	6.1	0.9	0	0	0
Hydrophilic		Threonine [T]	0	0	0	0	0	0	0	0	0.9	0.9	0	0
Hydrophobic		Valine [V]	6.0	35.7	0	0	53.0	0	0	0	1.7	0	4.3	0
Hydrophobic		Tryptophan [W]	0	0	3.4	0	0	100.0	0	0	0	0	0	0
Hydrophilic		Tyrosine [Y]	0	0	11.2	0	0	0	0	0	0	0	0	2.6
		Frequently modified residues	F/L	Ile/V	F/C/Y	P	Ile/N	W	A/S	G	E/Q	D/E	L	H

Supplementary Figure 12 Variation in the consensus motif identified in ALMT. Percentage residue frequency extracted from Supplementary Fig. 10. The numbers 1-12 correspond to the amino acid residues 213-224 from TaALMT1. The amino acids most common in these positions across the ALMT are shown in the bottom row of the table. The majority of amino acids in this region are hydrophobic (indicated by red colour).

Supplementary Table 1 Primers used for cloning and site directed mutagenesis.

Gene ID	Primer name	Primer sequence	Use
DQ072260	TaALMT1_F	5' ATGGATATTGATCACGGCAGAG 3'	Cloning and Sequencing
	TaALMT1_R	5' TTACAAAATAACCACGTCAGGCAAAGG 3'	
	TaMut1_F (F213C)	5' TGCACCACCGTgTgCCTCTTCCCCG 3'	Mutagenising F213 to C in pGEM-HE
	TaMut1_R (F213C)	5' CGGGGAAGAGGcAcACGGTGGTGCA 3'	
	TaMut2_F (F213C,215C)	5' CACCACCGTgTgCCTCTgCCCCGTCTGGG 3'	Mutagenising F213 and F215 to C in pGEMHE-D
	TaMut2_R (F213C,215C)	5' CCCAGACGGGGcAGAGGcAcACGGTGGTG 3'	
	TaMut3_F (F215C)	5' GTCTTCCTCTgCCCCGTCTGG 3'	Mutagenising F215 to C in pGEMHE-D
	TaMut3_R (F215C)	5' CCAGACGGGGcAGAGGAAGAC 3'	
XM_002275959	VvALMT9_F	5' ATGACCGCGAAACTTGGGTCG 3'	Cloning and Sequencing
	VvALMT9_R	5' CTACAACTTCAGCCACCTGCGC 3'	
	Vv9Mut1_F (Y237C)	5' TATGTTTGCTTGTAAATATATGCATCTGTCCCATCT 3'	Mutagenising Y237 to C in pGEMHE-D
	Vv9Mut1_R (Y237C)	5' CAGCCCAGATGGGACAGATGCATATATTTAC 3'	
AT1G08430	AtALMT1_F	5' ATGGAGAAAGTGAGAGAGATAGTGAG 3'	Sequencing
	AtALMT1_R	5' TTACTGAAGATGCCCATTACTTAATG 3'	
EF424084	HvALMT1_F	5' ATGGAGGTTGATCACCGCATC 3'	Cloning
	HvALMT1_R	5' TCAACTCGCAATGTTGATAGCG 3'	
AT5G46600	AtALMT13_F	5' ATGGGGTACAAGGTCGAAGC 3'	Cloning
	AtALMT13_R	5' CTAGATTAACCGGTTCTCAATTCG 3'	
AT5G46610	AtALMT14_F	5' ATGTCGGACAGGGTCCATGA 3'	Cloning
	AtALMT14_R	5' CCTAGTGGTT GGCGTGGAGTGA 3'	
Os04g0417000	OsALMT5_F	5' GGATCCATGCAATCTGCTGCCGTG 3'	Cloning
	OsALMT5_R	5' CCCGGGTCAACTCATGATGTTGATA 3'	
Os10g0572100	OsALMT9_F	5' GGATCCATGGCTTGTGCTCCAGA 3'	Cloning
	OsALMT9_R	5' CCCGGGTTACTCAGCTGCAGTGGAA 3'	