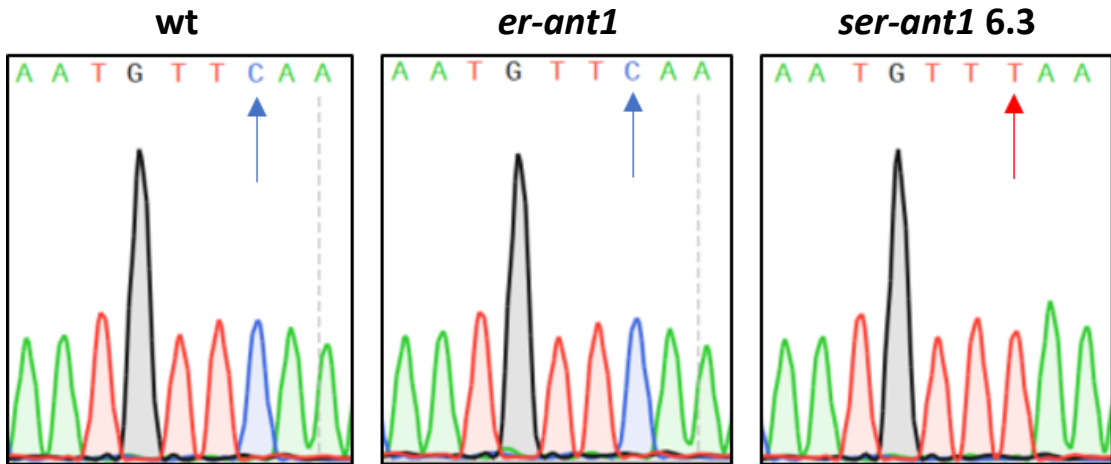


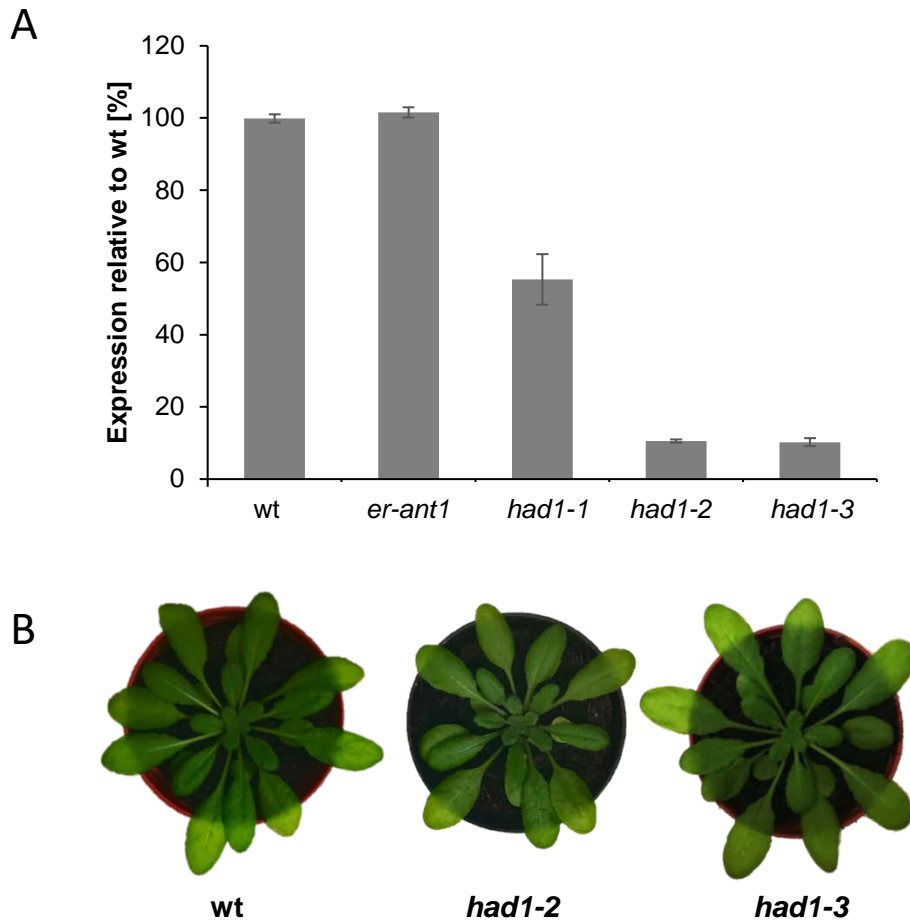
**Supplemental Figure S1: SNP induced changes in the *At2g33255* gene product.**

The individual positions in the amino acid sequence (taken from BLAST: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) are indicated by red arrows. The resulting amino acid changes and photos of representative plants are displayed above the arrows. Stop codon (\*). Plants were grown under standard growth conditions (diurnally for 10 h at 22°C and 120  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  light intensity followed by 14 h at 18°C without light, CO<sub>2</sub> levels around 400 ppm).



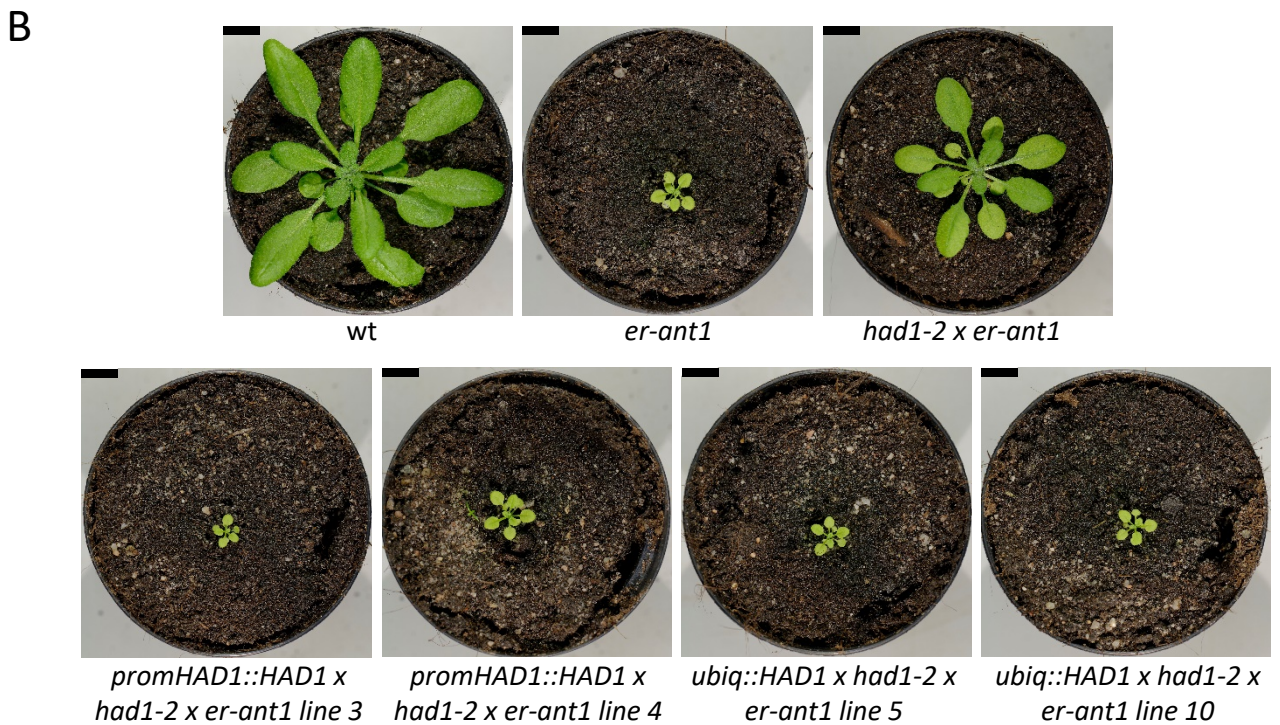
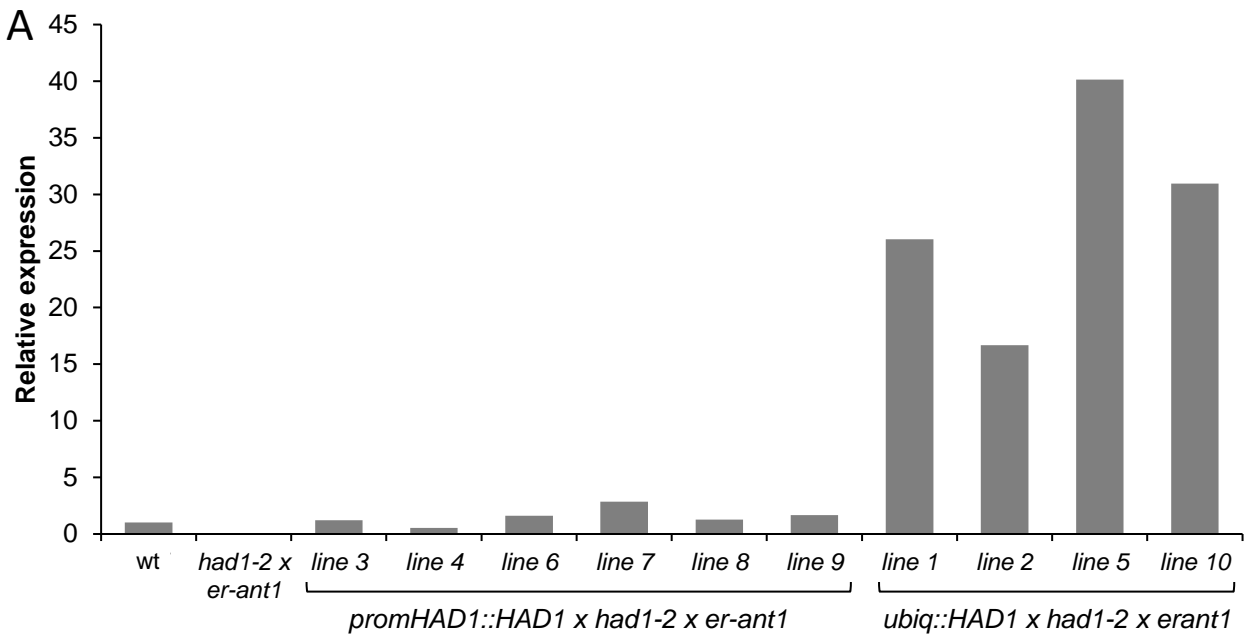
**Supplemental Figure S2: Confirmation of the candidate SNP in *At2g33255* of *ser-ant1* line 6.3.**

Comparison of the sequences determined by Sanger sequencing of genomic DNA (gDNA) from the wild type (wt), *er-ant1* and the *ser-ant1* M<sub>3</sub> line 6.3. Blue arrows highlight nucleotides identical to the reference genome at TAIR10. The red arrow highlights the confirmed candidate single nucleotide polymorphism (SNP).



**Supplemental Figure S3: Analysis of *At2g33255* T-DNA insertion lines.**

(A) Expression of *At2g33255* in *er-ant1* and three *had* transfer-DNA (T-DNA) insertion lines relative to the wild type (wt). Expression levels were determined by qRT-PCR. RNA for cDNA synthesis was extracted from leaves of three weeks old plants. Data were normalized to the SAND (*At2g28390*) housekeeping gene. Shown are mean values of the expression in at least four plants  $\pm$  SE. Line *had1-1* still contains about 50% of the transcript whereas the level in *had1-2* and *had1-3* is at the detection limit. Therefore, we considered *had1-1* as a knock-down and *had1-2* and *had1-3* as knock-out plants. (B) Phenotype of four weeks old plants grown under standard growth conditions (diurnally for 10 h at 22°C and 120  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  light intensity followed by 14 h at 18°C without light, CO<sub>2</sub> levels around 400 ppm). Images of panel B were digitally extracted for comparison.



**Supplemental Figure S4: Analysis of *promHAD1::HAD1 x had1-2 x er-ant1* and *ubiq::HAD1 x had1-2 x erant1* lines.**

(A) Expression of *At2g33255* in various mutant lines relative to the wild type (wt). Expression levels were determined via RT-qPCR. RNA for cDNA synthesis was extracted from leaves of 3 weeks old plants. Data were normalised to the ubiquitin housekeeping gene (*At4g05320*). (B) Phenotype of four weeks old plants. Plants were grown for 4 weeks under standard growth conditions (diurnally for 10 h at 22°C and 120  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  light intensity followed by 14 h at 18°C without light, CO<sub>2</sub> levels around 400 ppm). Black bars represent 10 mm.

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      *           20           *           40           *           60
NtPLPP1 : MIGRCVSAQSSKPNELKFQTLNGIQELAE TRRFKAWFLDQFGVLDHDKQYPYGAISTLEKLAT : 63
At2g33255 : -----MTFLLSRTEISLTLRPSCSISMAN----- : 24

      *           80           *           100          *           120
NtPLPP1 : YGAKMVIIVSNSSRRASTTLEKLRSLGFDPSLFIGAITSGELTHQYLQRRDDAWFASLGRSCIH : 126
At2g33255 : -----LITNAKTRLRGVVFDMDGTLTVPVLDFAAMYRAVLGEDAYKRIRKAESPSTGIDILHHI : 81

      *           140          *           160          *           180
NtPLPP1 : MTWSDRGAISLESGLLEVVENAQGADFILAHGTEALGLSSGAALPMKLLDLEKILEQCATKKI : 189
At2g33255 : ESWSP----DKQQKAYEIIADYE-----KQGIDKLIQIMPETAELCGFIDSKKIKRGLITRNV : 134

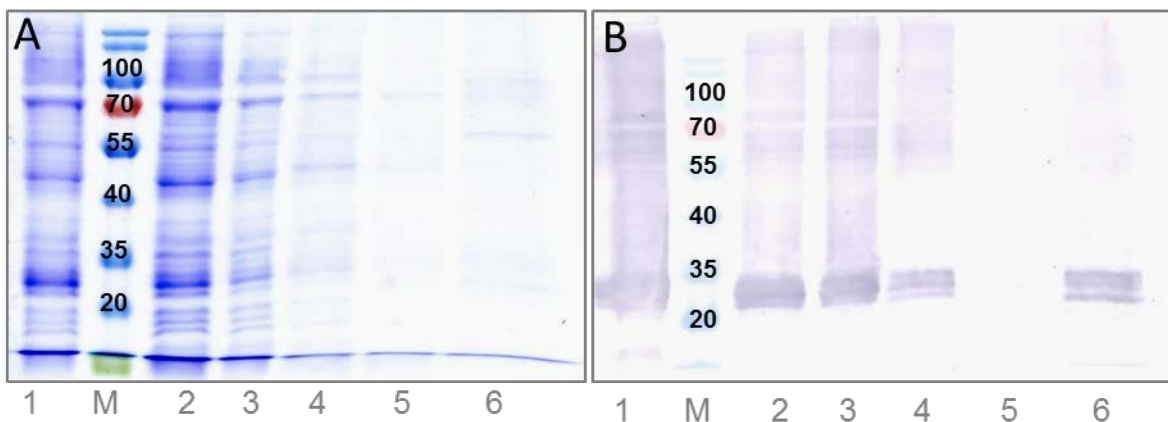
      *           200          *           220          *           240          *
NtPLPP1 : PMVWANPDFVTVFVRLRVMPGTLAATYEKLGGEVKWMGKPKDIIYKSAMKMAAEDASDCIAT : 252
At2g33255 : QKAID----IFHQRFEVIFSPALGREFRPYK-----PNPDPLLHICST--WDIQPNEVMMV : 184

      260           *           280           *           300           *
NtPLPP1 : GDSLHHDIKGANAAGIASAFITCGIHATELGLDKFGEVADDNSIHALALQNNAHPTYVIPSFT : 315
At2g33255 : GDSLKDDIACGKRAGAFICLLDETGRYGPDDFSVSLQPDFKVDLSKIQNLLETNFDLNP-- : 245
IV

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**Supplemental Figure S5: ClustalW alignment of the amino acid sequences of the pyridoxal 5'-phosphate phosphatase (PLPP) from *Nicotiana tabacum* (NtPLPP1) and the *At2g33255* gene product.**

Residues identical among the two sequences or with similar properties are indicated by black shading. Residues not identical are highlighted by different shading (gray/white). The red box marks the conserved haloacid dehalogenase-like superfamily (HADSf) motif IV (E/DD, GDxxxD, or GDxxxxD). Dashes represent introduced gaps for alignment improvement. Numbers at the right indicate amino acid positions.



**Supplemental Figure S6: Purification of the HAD-type hydrolase, At2g33255, heterologously expressed in *Escherichia coli* Rosetta cells.**

(A) Coomassie stained SDS-PAGE of the purification of the haloacid dehalogenase (HAD)-type hydrolase. Lane 1: Lysate of induced *E. coli* cells; Lane M: Protein marker PAGERuler (ThermoFisher), the numbers represent the molecular masses (given in kDa); Lane 2: soluble protein fraction after centrifugation (5000 g, 4°C); Lane 3: flow through of Immobilized Metal Ion Affinity Chromatography (IMAC); Lane 4: flow through of the first washing step with binding buffer; Lane 5: flow through of the second washing step with washing buffer; Lane 6: eluate. The calculated molecular mass of the HAD-type hydrolase is 27.5 kDa (<http://aramemnon.uni-koeln.de>). (B) Immunochemical staining of the same samples as in (A) with the anti-His antibody and the corresponding secondary anti-mouse antibody (alkaline phosphatase conjugate). Colorimetric detection was by NBT/BCIP staining.

**Supplemental Table S1: SNP in the coding sequence of *At2g33255* from different *er-ant1* suppressor lines.**

Line	AF	Max AF	SNP Position	AA change
8.3	0.86	0.88	14098898	G→D
4.1	0.97	1	14099659	G→E
4.2	0.83	0.94	14099659	G→E
4.4	1	1	14099659	G→E
6.3	0.94	0.97	14099679	Q→*
5.6	0.86	0.92	14100204	G→E
17.1	0.98	0.98	14100204	G→E

Shown are allele frequencies (AF) of the single nucleotide polymorphisms (SNPs) in *At2g33255*, highest allele frequency among all candidate SNPs in the respective line (Max AF), *At2g33255* SNP position on chromosome 2 (SNP position), and the respective amino acid (AA) change caused by the SNP. Lines are sorted by SNP position (5'→3'). Redundant SNPs are highlighted by identical colours.