SUPPLEMENTARY MATERIALS

Gain-of-function mutations of *AtNHX1* suppress *sos1* salt sensitivity and improve salt tolerance in Arabidopsis

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Table S1. Primers used for site-directed mutagenesis.

***sup* Mutant**	**Primer Name**	**Primer Sequence (5' to 3')**
sup1604	c65t	5'-atcacgcttctgtggttgtgttgaatctctttgttgc-3'
	c65t_antisense	5'-gcaacaaagagattcaacacaaccacagaagcgtgat-3'
sup806	c701t	5'-gcaaccggtctgataagtgtgtatgttatcaagaagcta-3'
	c701t_antisense	5'-tagcttcttgataacatacacacttatcagaccggttgc-3'
sup610	g748a	5'-ggaaggcactcaactgaccgaaaggttgccctta-3'
	g748a_antisense	5'-taagggcaacctttcggtcagttgagtgccttcc-3'
sup606	c757t	5'-ctgaccgagaggttgcctttatgatgcttatggcg-3'
	c757t_antisense	5'-cgccataagcatcataaaggcaacctctcggtcag-3'
sup810	c875t	5'-ccattacacatggcacaatgtaatggagagctcaag-3'
	c875t_antisense	5'-cttgagctctccattacattgtgccatgtgtaatgg-3'
sup1602	c1481t	5'-cttcttgacacggctcactcgaaccgtgc-3'
	c1481t_antisense	5'-gcacggttcgagtgagccgtgtcaagaag-3'
sup908	c1534t	5'-ttgatgactccttcatgcgatccgtctttggagg-3'
	c1534t_antisense	5'-cctccaaagacggatcgcatgaaggagtcatcaa-3'

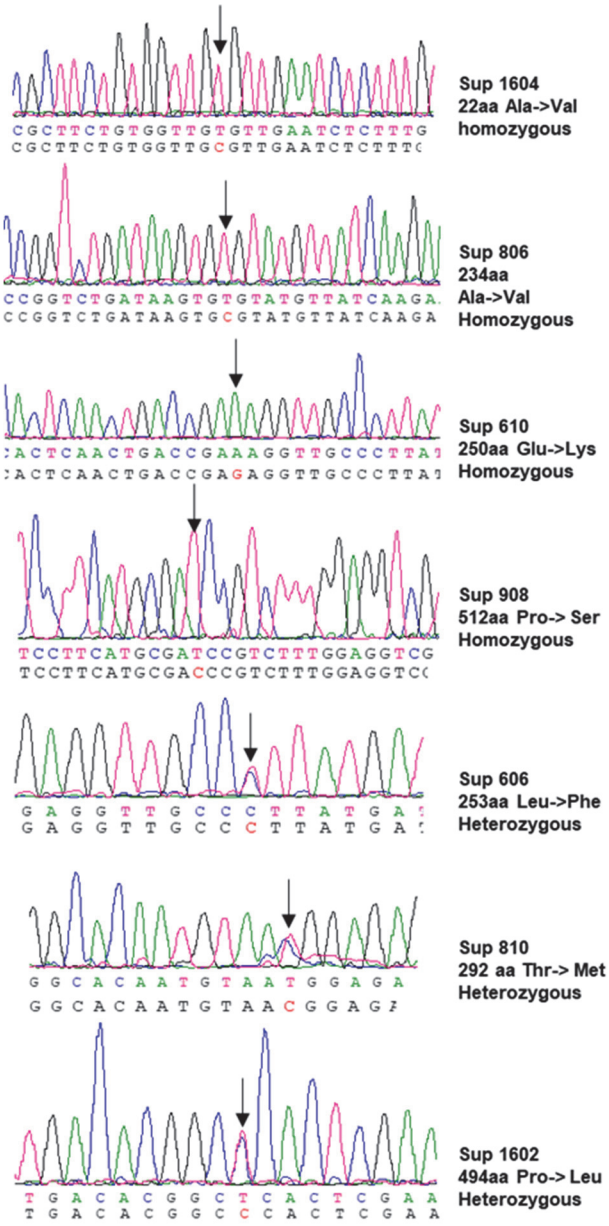

Fig. S1. Sequencing chromatogram of the identified *sup AtNHX1* mutants. The zygosity of the *sup* mutants were identified using their respective sequencing chromatograms. Arrows indicate the mutated nucleotides.

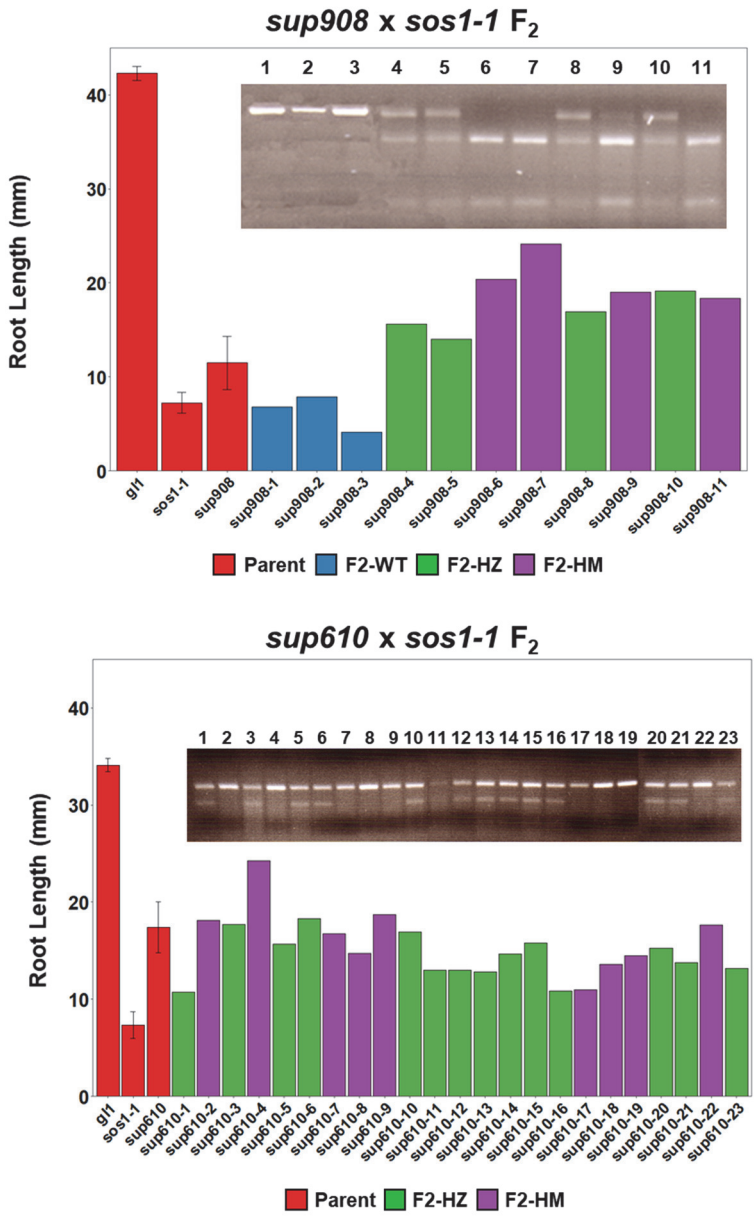

Fig. S2. Genotype and phenotype segregation in the F_2 of the genetic crosses between *sos1-1* and the *sup908* and *610* mutants, verifying the dominant inheritance pattern of the mutations. The inserts show the genotypes of the F_2 seedlings identified with cleaved amplified polymorphic sequence (CAPS) markers. The *sup908* allele is identified through its cleavage by Sau3AI, which is absent in the wild type, while the *sup610* allele is identified through its non-cleavage by MnlI. Root lengths of individual seedlings are shown with color-coded bars according to their zygosity for their *AtNHX1* allele.

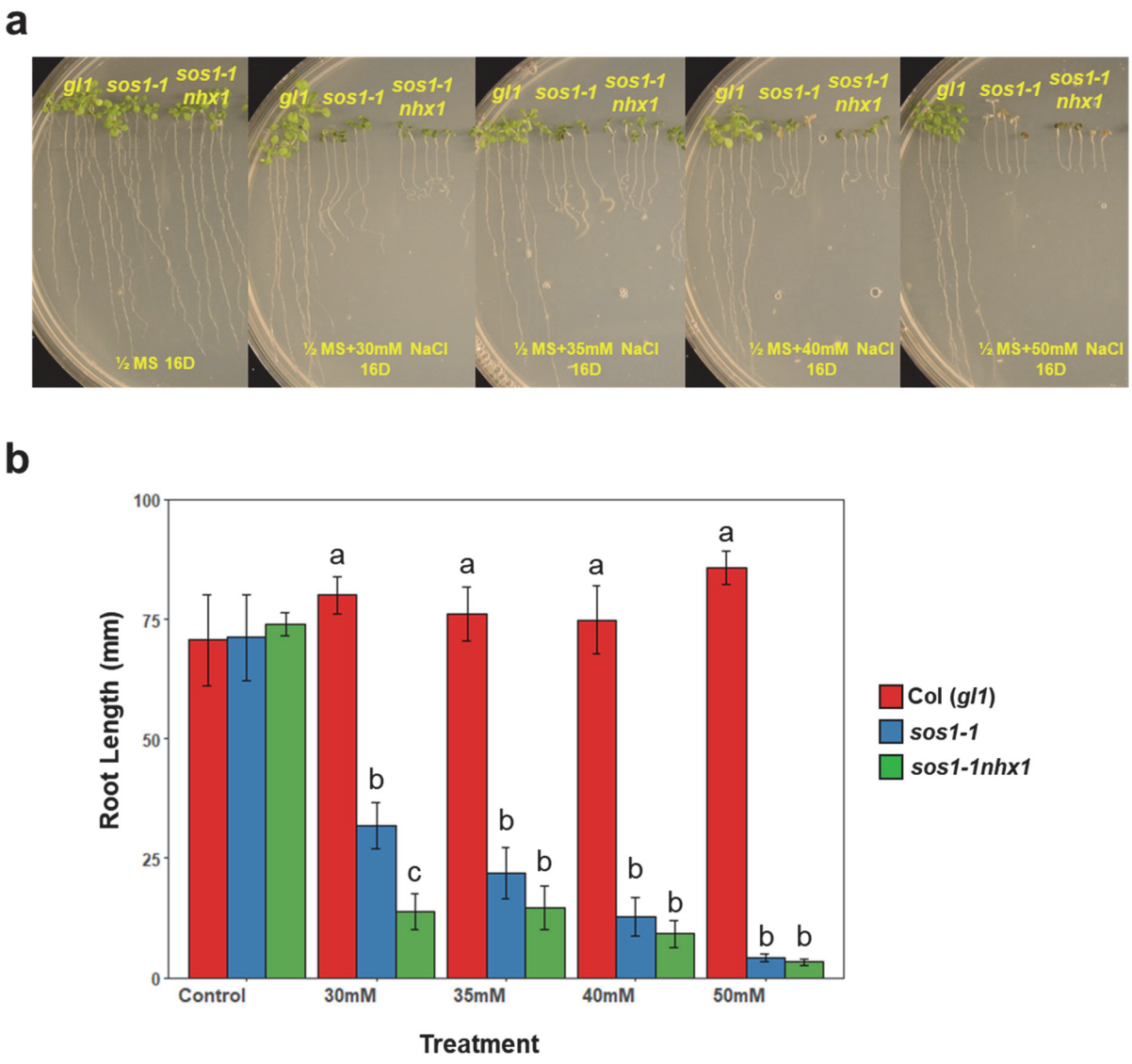

Fig. S3. Growth phenotype of the *sos1nhx1* double mutant in response to salt stress. a Root growth assay of wild type Col (*gl1*), *sos1-1* and *sos1nhx1*. The *sos1nhx1* double mutant was generated by crossing *sos1-1* with the T-DNA insertion *atnhx1* mutant allele. **b** Quantitative measurement of root lengths of these three genotypes in response to different concentrations of NaCl. Bars represent means, and error bars represent standard errors (n ≥ 3). Statistical significance among genotypes within treatments was computed with ANOVA and Tukey's post-hoc HSD test ($P < 0.05$) and different statistical groups are represented by letters.

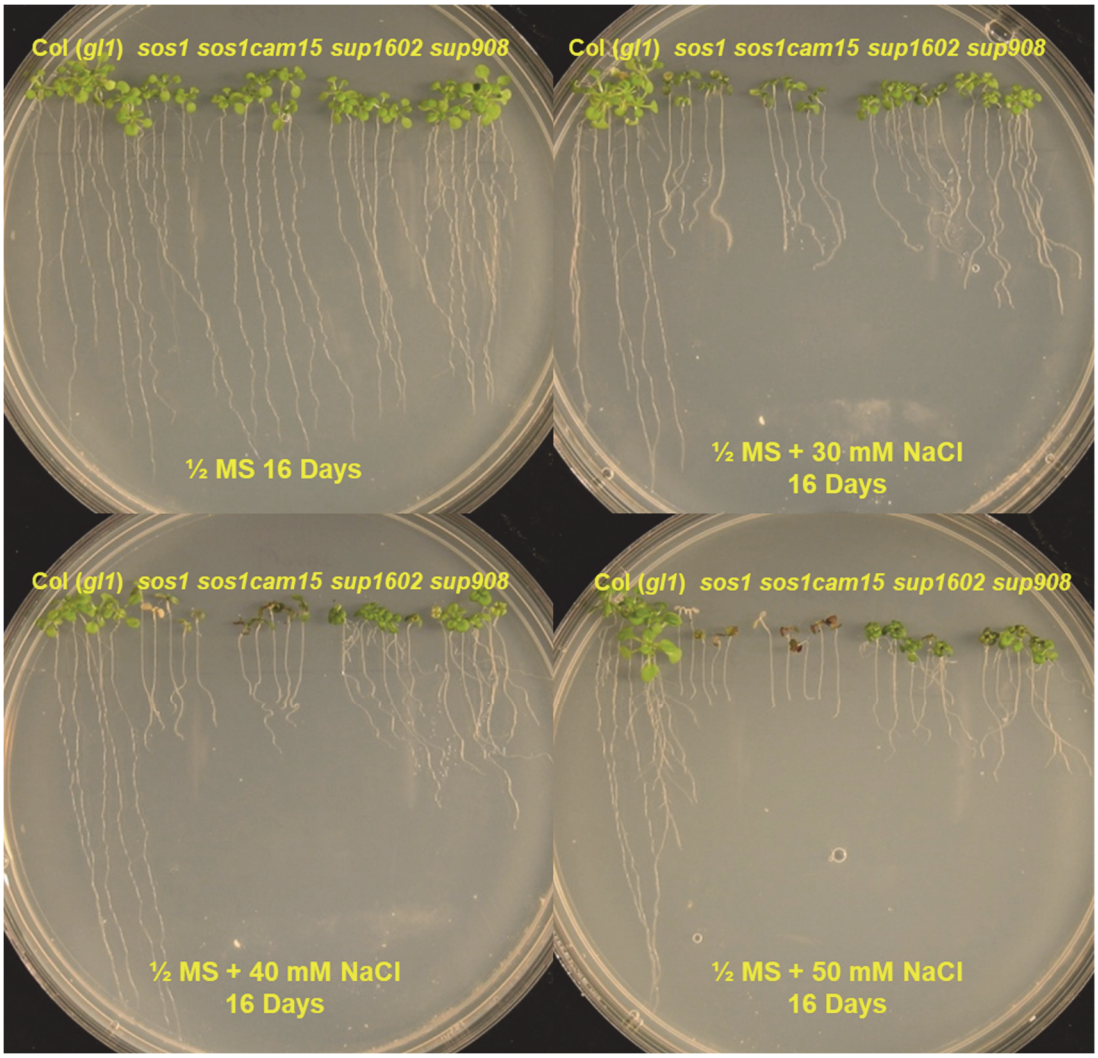

Fig. S4. Growth phenotype of *sos1cam15, sup1602,* and *sup908* in response to salt stress. The *sos1cam15* double mutant was obtained by crossing *sos1-1* with the T-DNA insertion mutant *cam15*. The suppressor mutants *sup1602* and *908* were used as controls showing suppression of *sos1-1* salt sensitivity, while the *cam15* mutation did not suppress *sos1-1* salt sensitive phenotype.

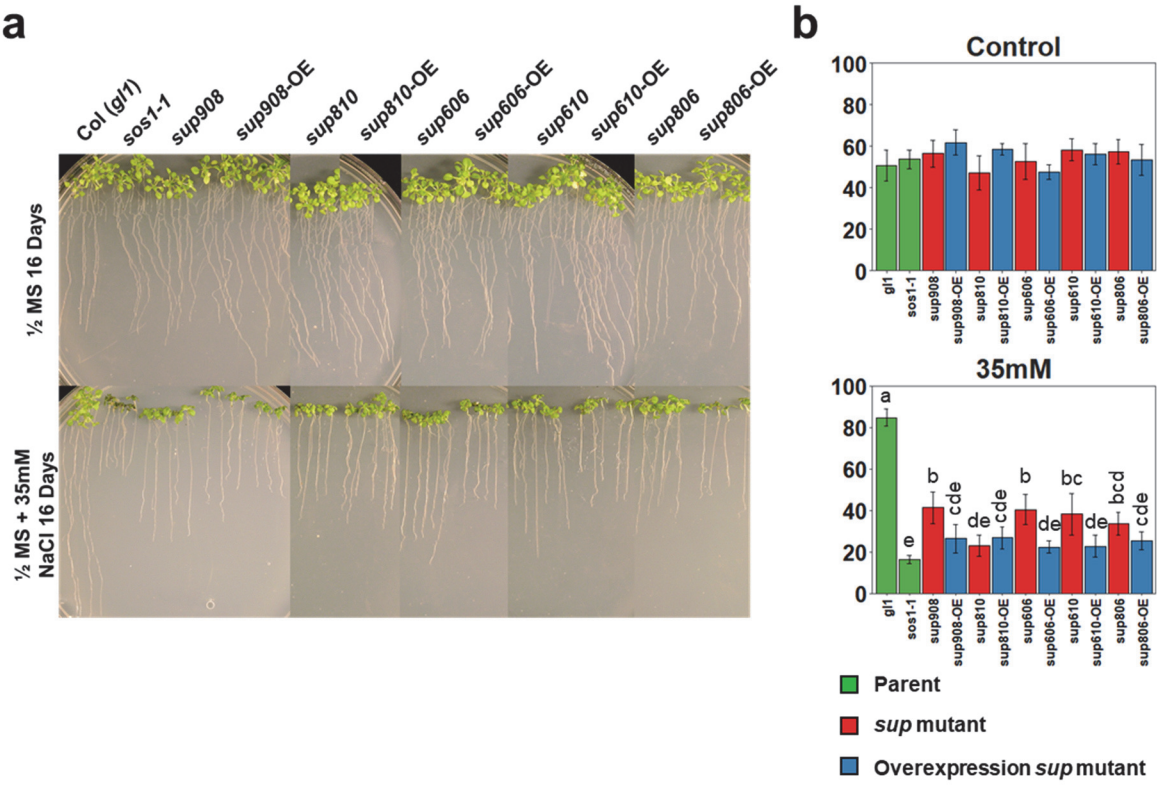

Fig. S5. Overexpression of the *sup* alleles showed less suppression of *sos1-1* salt sensitivity than the native *sup* alleles. a Root growth assay of wild type, *sos1-1*, *sup* mutants and *sup*-OE seedlings. The *sup*-OE genotypes are the transgenic plants overexpressing the *AtNHX1* alleles in the *sos1-1* genetic background using the 35S promoter driven the ORF of the *AtNHX1* alleles. *AtNHX1* ORF cloning and site-directed mutagenesis to generate the *sup* *AtNHX1* alleles were described in "Materials and Methods". The ORFs of *AtNHX1* alleles were recombined into the plant expression vector pEarleyGate 100 (Earley et al. 2006), and the constructs were transformed into *sos1-1* mutant to generate *sup*-OE plants using the floral dip method (Clough and Bent 1998). Homozygous transgenic lines were selected for salt sensitivity test. **b** Quantitative measurement of the root lengths. Bars represent mean root lengths, and error bars represent standard errors (n = 5). Statistical significance among genotypes within a treatment group was computed with ANOVA and Tukey's post-hoc HSD test ($P < 0.05$, n = 5) and different statistical groups are represented by letters.

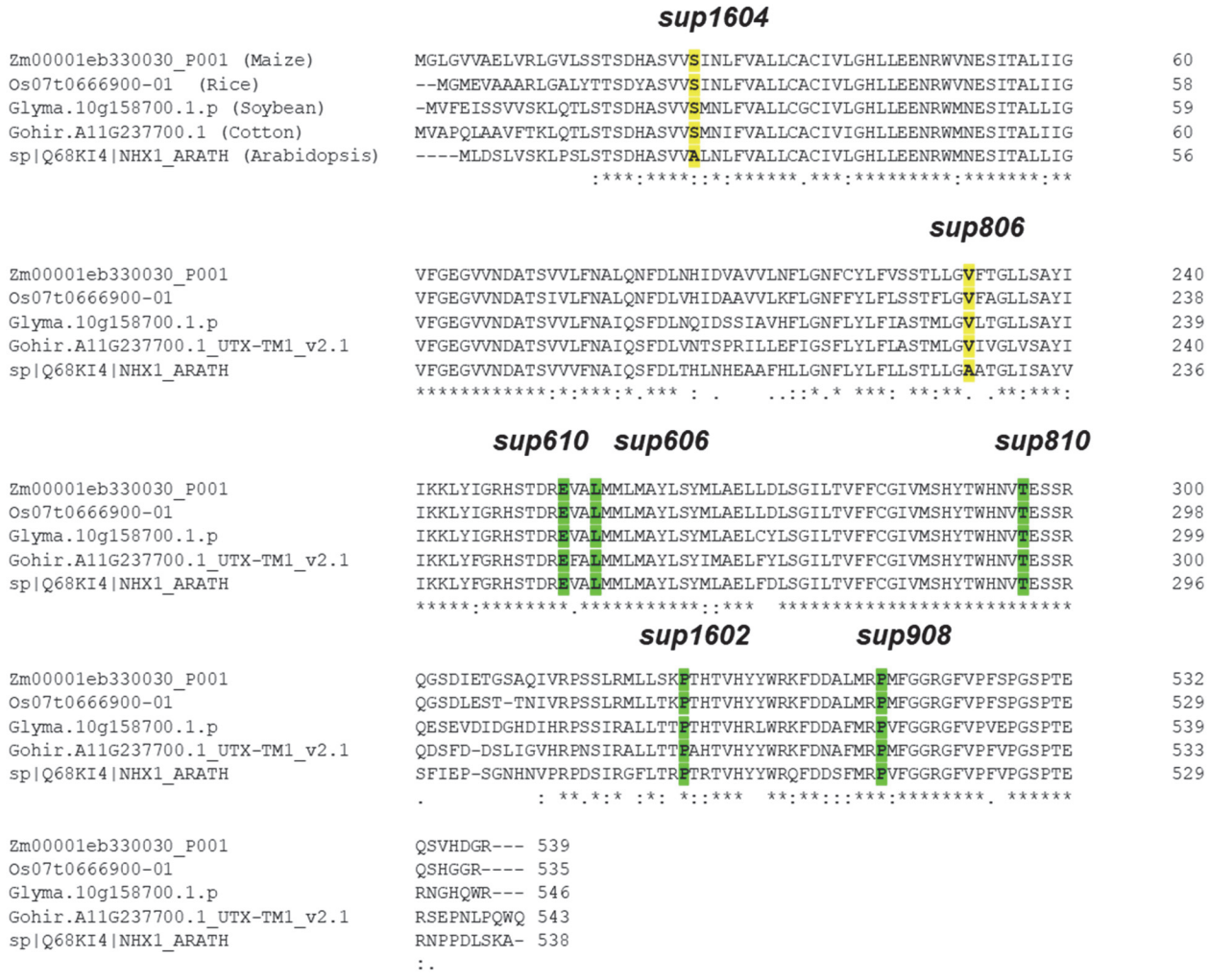

Fig. S6. Partial sequence alignment of NHX1 homologs in Arabidopsis and major crop species. NHX1 homologs in major crop species (maize, rice, soybean, and cotton) were aligned and compared to the Arabidopsis AtNHX1 protein. Residues wherein mutations were identified in the *sup* lines are highlighted. Residues in yellow are not conserved in the other crop species compared to Arabidopsis, while residues in green are conserved in all species shown.