

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.

<i>sup</i> Mutant	Primer Name	Primer Sequence (5' to 3')
<i>sup1604</i>	c65t	5'-atcacgcttctgtggttggtgaatctctttgttgc-3'
	c65t_antisense	5'-gcaacaaagagattcaacacaaccacagaagcgtgat-3'
<i>sup806</i>	c701t	5'-gcaaccggtctgataagtgtgtatggtatcaagaagcta-3'
	c701t_antisense	5'-tagcttctgataacatacacacttatcagaccggttgc-3'
<i>sup610</i>	g748a	5'-ggaaggcactcaactgaccgaaagggtgcctta-3'
	g748a_antisense	5'-taagggcaaccttcggtcagttgagtgccttc-3'
<i>sup606</i>	c757t	5'-ctgaccgagaggttgcctttatgatgcttatggcg-3'
	c757t_antisense	5'-cgccataagcatcataaaggcaacctctcggtcag-3'
<i>sup810</i>	c875t	5'-ccattacacatggcacaatgtaatggagagctcaag-3'
	c875t_antisense	5'-cttgagctctccattacattgtgcatgtgtaatgg-3'
<i>sup1602</i>	c1481t	5'-cttcttgacacggctcactcgaaccgtgc-3'
	c1481t_antisense	5'-gcacggttcgagtgagccgtgtcaagaag-3'
<i>sup908</i>	c1534t	5'-ttgatgactccttcatgcatccgtctttggagg-3'
	c1534t_antisense	5'-cctcaaagacggatcgcatgaaggagtcataa-3'

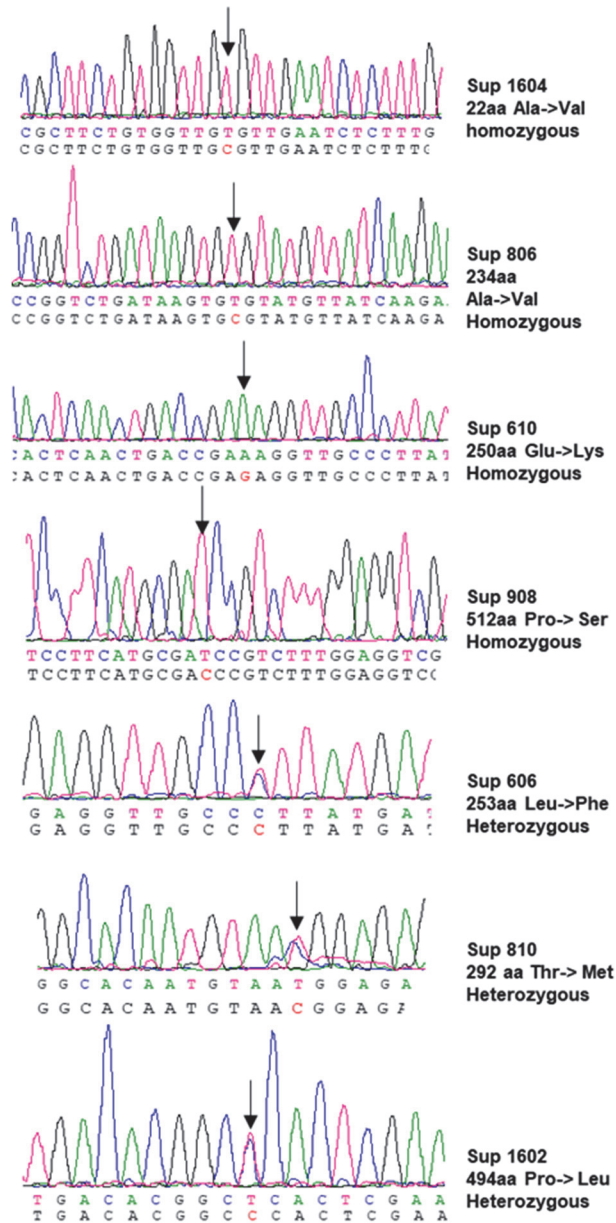


Fig. S1. Sequencing chromatogram of the identified *sup AtNHX1* mutants. The zygosity of the *sup* mutants were identified using their respective sequencing chromatograms. Arrows indicate the mutated nucleotides.

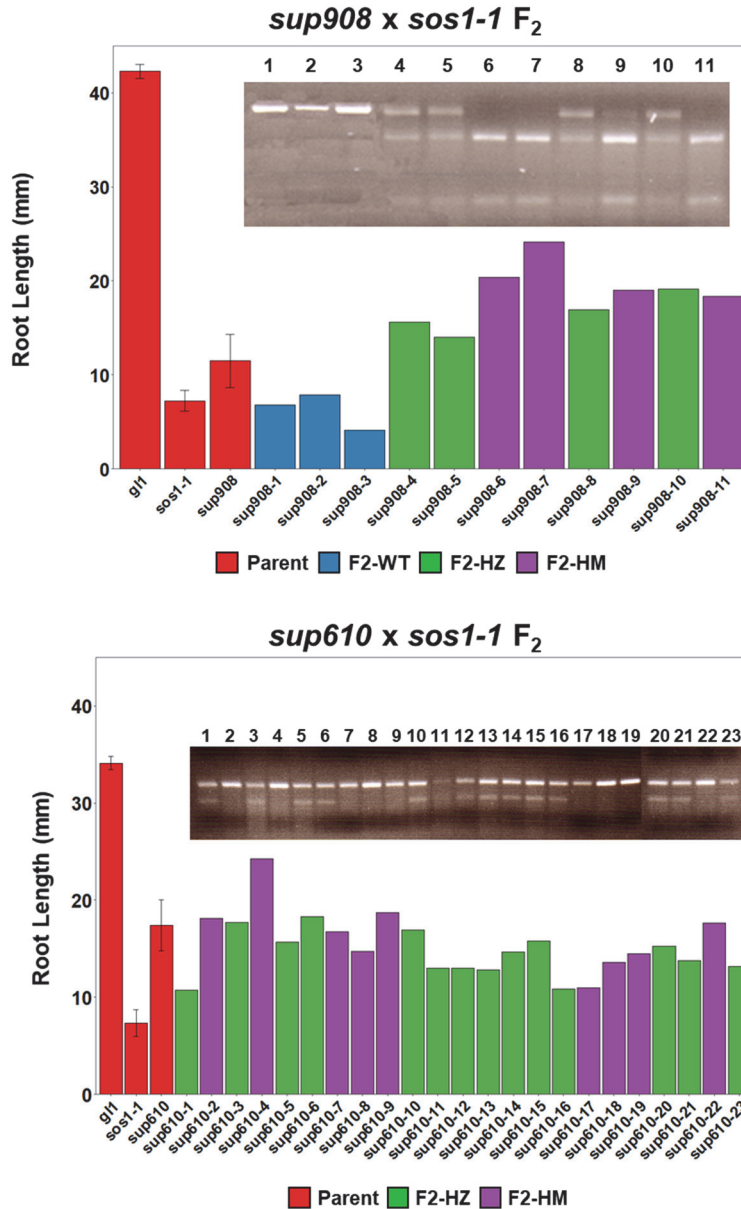


Fig. S2. Genotype and phenotype segregation in the F₂ of the genetic crosses between *sos1-1* and the *sup908* and *610* mutants, verifying the dominant inheritance pattern of the mutations. The inserts show the genotypes of the F₂ seedlings identified with cleaved amplified polymorphic sequence (CAPS) 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.

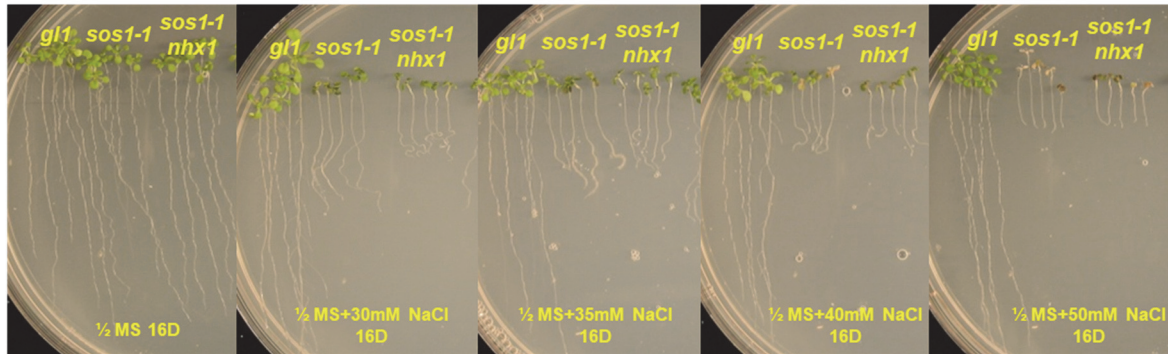
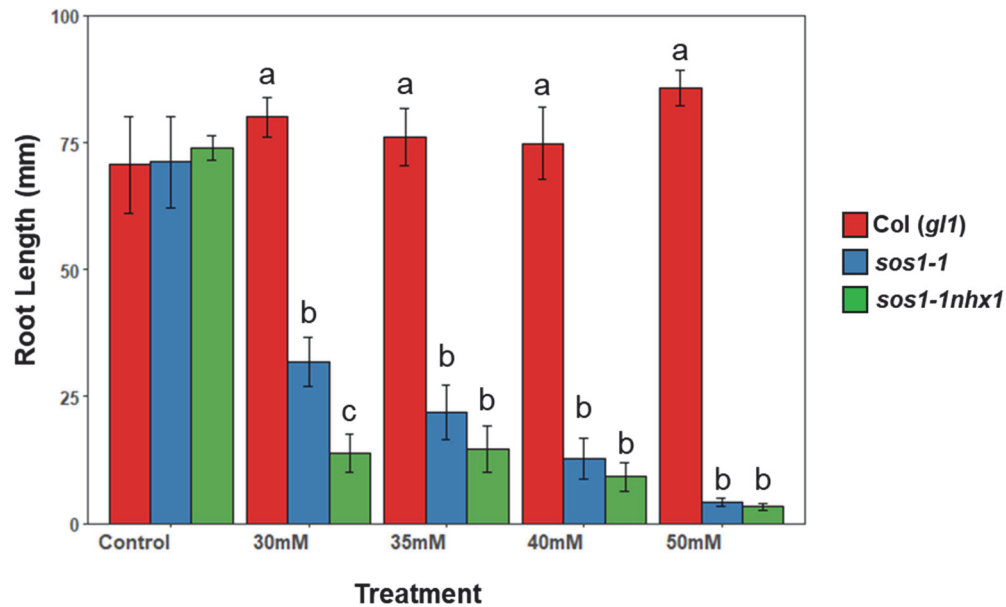
a**b**

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 ($n \geq 3$). 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.

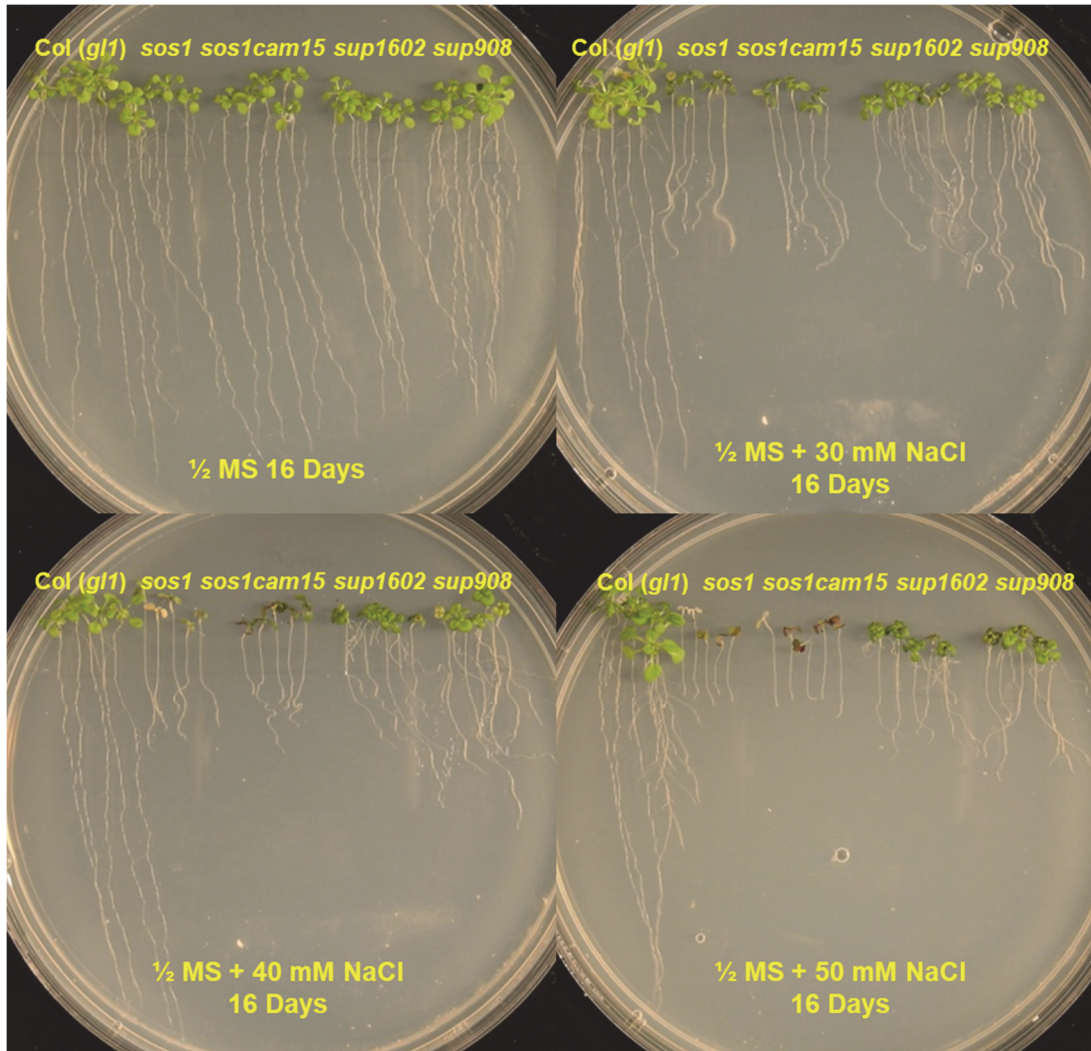


Fig. S4. Growth phenotype of *sos1cam15*, *sup1602*, and *sup908* in response to salt stress. The *sos1cam15* double mutant was obtained by crossing *sos1-1* with the T-DNA insertion mutant *cam15*. The suppressor mutants *sup1602* and *908* were used as controls showing suppression of *sos1-1* salt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

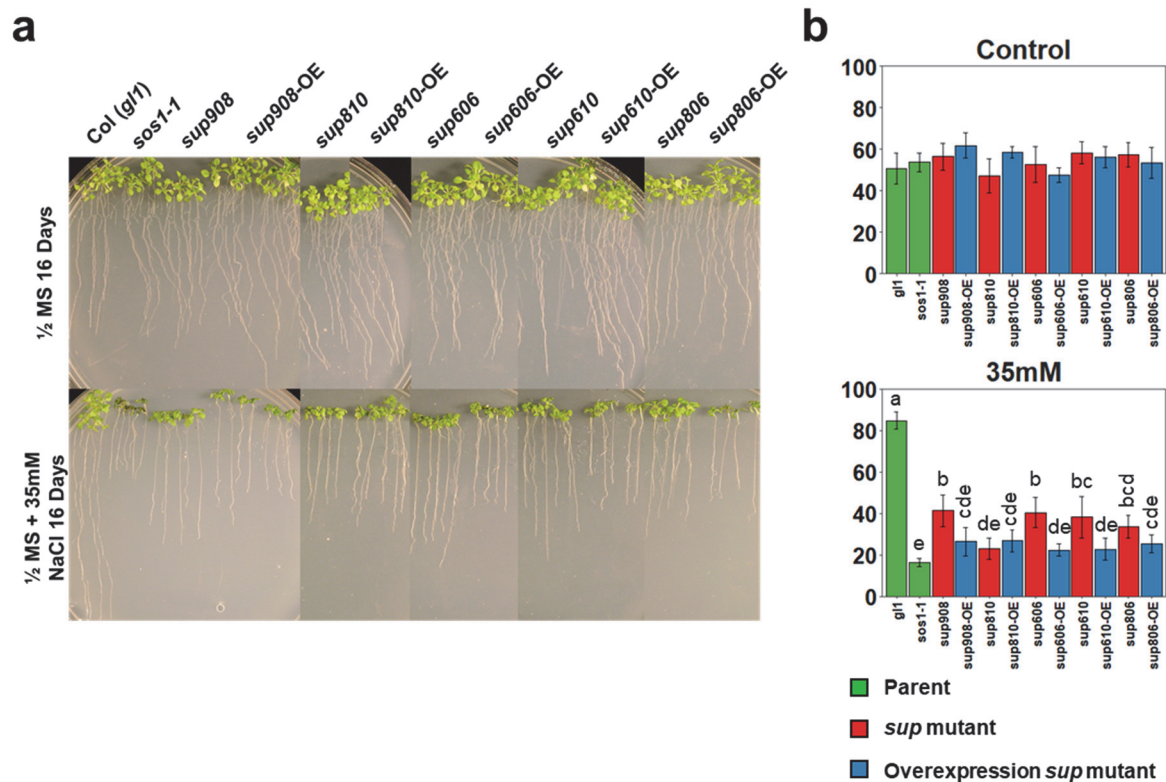


Fig. S5. Overexpression of the *sup* alleles showed less suppression of *sos1-1* salt sensitivity than the native *sup* alleles. **a Root growth assay of wild type, *sos1-1*, *sup* mutants and *sup*-OE seedlings. The *sup*-OE genotypes are the transgenic plants overexpressing the *AtNHX1* alleles in 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.**

sup1604				
Zm00001eb330030_P001 (Maize)	MGLGVVAELVRLGVLSSDSDHASVVS	INLFLVALLCACIVLGHLLLENRWNESITALIIG	60	
Os07t0666900-01 (Rice)	--MGMEVVAARLGALYTTSDYASVVS	INLFLVALLCACIVLGHLLLENRWNESITALIIG	58	
Glyma.10g158700.1.p (Soybean)	-MVFEISSVSKLQTLSTSDHASVVS	MNLFVALLCGCIVLGHLLLENRWNESITALIIG	59	
Gohir.A11G237700.1 (Cotton)	MVAPQLAAVFTKQLTLSTSDHASVVS	MNIFVALLCACIVIGHLLLENRWNESITALIIG	60	
sp Q68KI4 NHX1_ARATH (Arabidopsis)	----MLDSLVSKLPSLSTSDHASVVAL	NLFLVALLCACIVLGHLLLENRWNESITALIIG	56	
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sup806				
Zm00001eb330030_P001	VFGEGVNDATSVVLFNALQNF	LDLHIDAVVNLFLGNFCYLFVSSTLLGVFTGLLSAYI	240	
Os07t0666900-01	VFGEGVNDATSI	VLFNALQNFDLVHIDA	238	
Glyma.10g158700.1.p	VFGEGVNDATSVVLFNAIQSF	DLNQIDSSIAVHFLGNFLYLFI	239	
Gohir.A11G237700.1_UTX-TM1_v2.1	VFGEGVNDATSVVLFNAIQSF	DLVNTSPRILLEFIGSFLYLFI	240	
sp Q68KI4 NHX1_ARATH	VFGEGVNDATSVVVFNAIQSF	DLTHLNHEAAFHLLGNFLYLFI	236	
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sup610 sup606		sup810		
Zm00001eb330030_P001	IKKLYIGRHSTDR	VA	LMMLMAYLSYMLAELLDL	300
Os07t0666900-01	IKKLYIGRHSTDR	VA	LMMLMAYLSYMLAELLDL	298
Glyma.10g158700.1.p	IKKLYIGRHSTDR	VA	LMMLMAYLSYMLAELCYL	299
Gohir.A11G237700.1_UTX-TM1_v2.1	IKKLYFGRHSTDR	VA	LMMLMAYLSYMAELFY	300
sp Q68KI4 NHX1_ARATH	IKKLYFGRHSTDR	VA	LMMLMAYLSYMLAELFDL	296
			*****:*****.*****:*** **	
sup1602		sup908		
Zm00001eb330030_P001	QGSDIETGSAQIVR	PSSLRMLLSK	THTVHYYWRKFDDALMR	532
Os07t0666900-01	QGSdleST-TNIVR	PSSLRMLLTK	THTVHYYWRKFDDALMR	529
Glyma.10g158700.1.p	QSEVDIDGHD	IHRPSSIRALLT	THTVHRLWRKFDDAFMR	539
Gohir.A11G237700.1_UTX-TM1_v2.1	QDSFD-DSLIGV	HRENSIRALLT	TAHTVHYYWRKFDNAFMR	533
sp Q68KI4 NHX1_ARATH	SFIEP-SGNHN	VPRPDSIRGFLTR	TRTVHYYWRQFDDSFMR	529
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Zm00001eb330030_P001	QSVHDGR---	539		
Os07t0666900-01	QSHGGR----	535		
Glyma.10g158700.1.p	RNGHQWR---	546		
Gohir.A11G237700.1_UTX-TM1_v2.1	RSEPNLPQWQ	543		
sp Q68KI4 NHX1_ARATH	RNPPDLSKA-	538		
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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.