Supplementary data

Figure S1

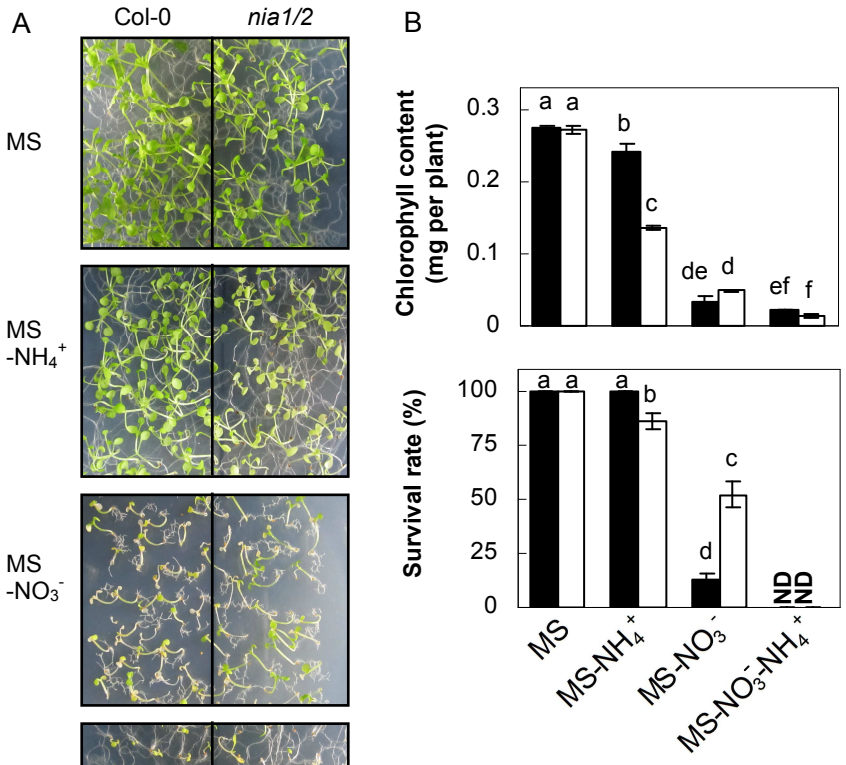

Figure. S1 Morphology (A), chlorophyll content and survival rate (B) of stratified seeds of wild-type (Col-0) and *nia1/2* grown in the MS medium with or without nitrate and ammonium as indicated for 20 d. Data are means ± SE from three independent experiments. Bars with different letters are significantly different at the $P < 0.05$ level according to multiple comparison.

Figure S2

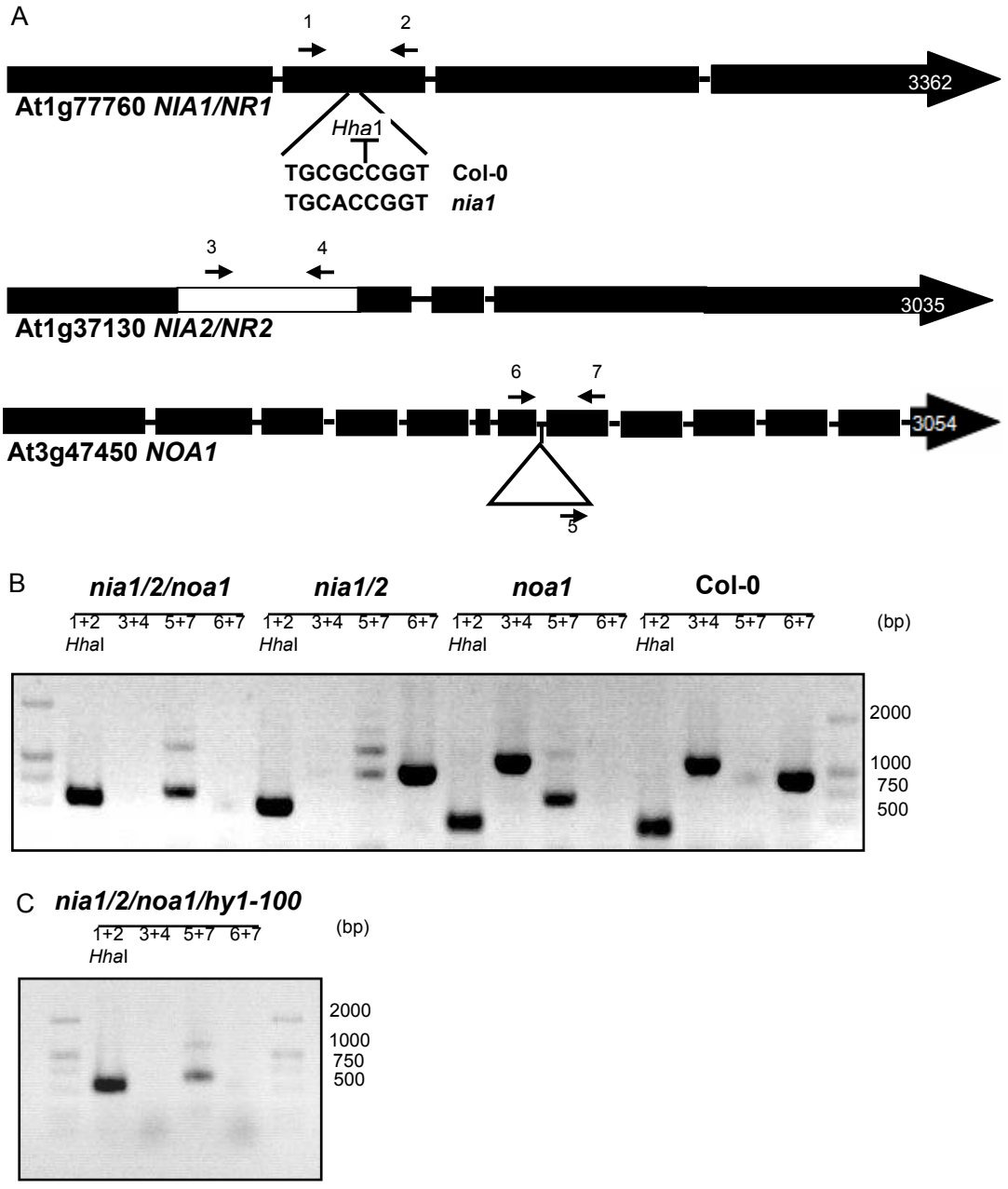

Figure S2. Genotyping of the generated *nia1/2/noa1* and *nia1/2/noa1/hy1-100* mutant. The position of the T-DNA insertion and primers (indicated with arrows and numbers) used for genotyping (Supplementary Table S1 at *JXB* online) mutations in the corresponding genes were shown in (A). The *Hha*I restriction site for genotyping of *nia1* mutations is also shown. PCR-based genotyping of wild-type, *noa1*, *nia1/2*, *nia1/2/noa1*,

and *nia1/2/noa1/hy1-100* mutant plants (B, C). The DNA of each genotypes was extracted and PCR-amplified using primer pairs 1+2, 3+4, 5+7, or 6+7, respectively.

Figure S3

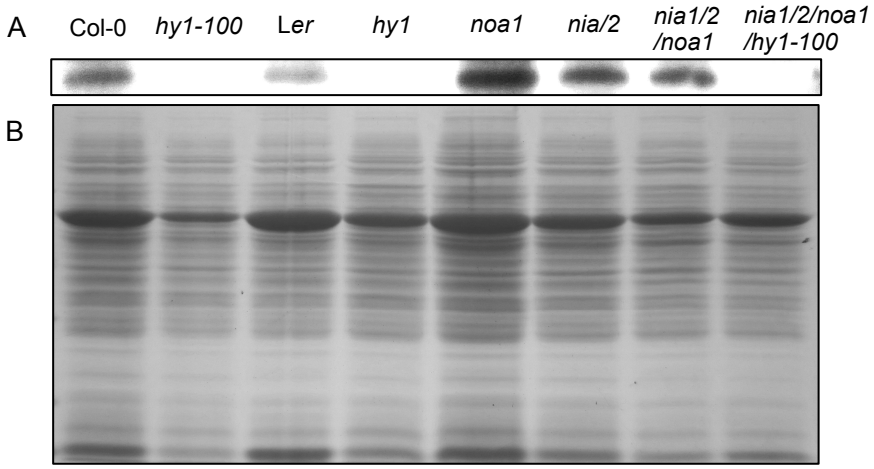

Figure S3. Detection of the HY1 protein level (A) in 5-d-old seedlings of Col-0, *hy1-100*, L*er*, *hy1*, *noa1*, *nia1/2*, *nia1/2/noa1*, *nia1/2/noa1/hy1-100* by western-blot analysis. Coomassie Brilliant Blue-stained gels (B) are present to show that equal amounts of proteins were loaded.

Figure S4

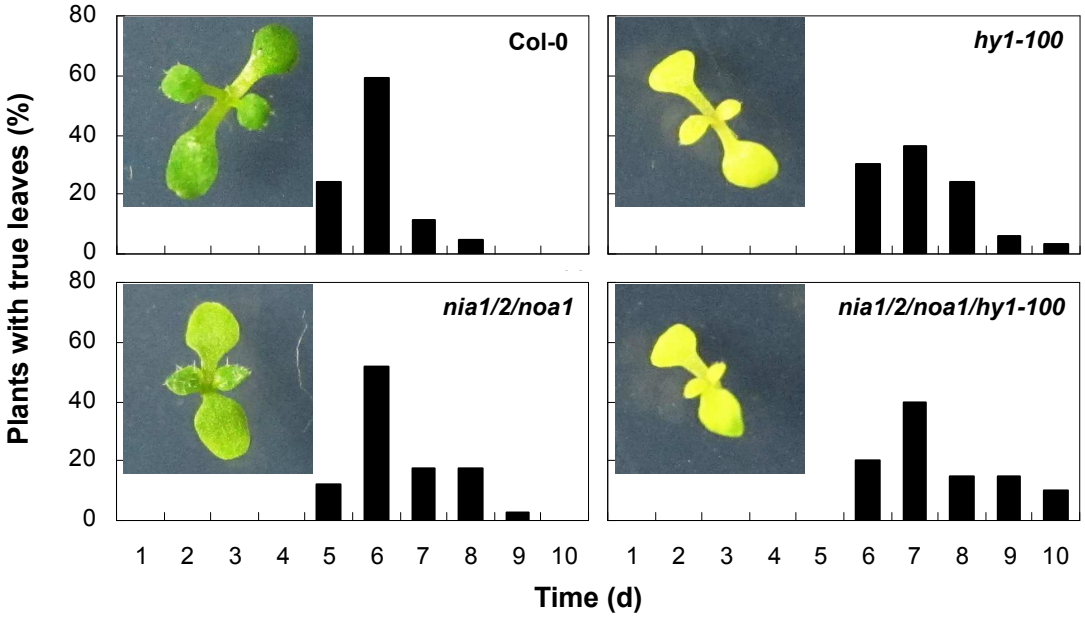

Figure S4. Developmental phenotypes of wild-type, *hy1-100*, *nia1/2/noa1*, and *nia1/2/noa1/hy1-100* plants at the early seedling stage. The percentage of seedlings from the indicated genotypes displaying their first pair of true leaves were scored daily from day 1 to 10 after sowing (n = 200). Growth phenotypes of corresponding plants grown for 8 d were also shown.

Figure S5

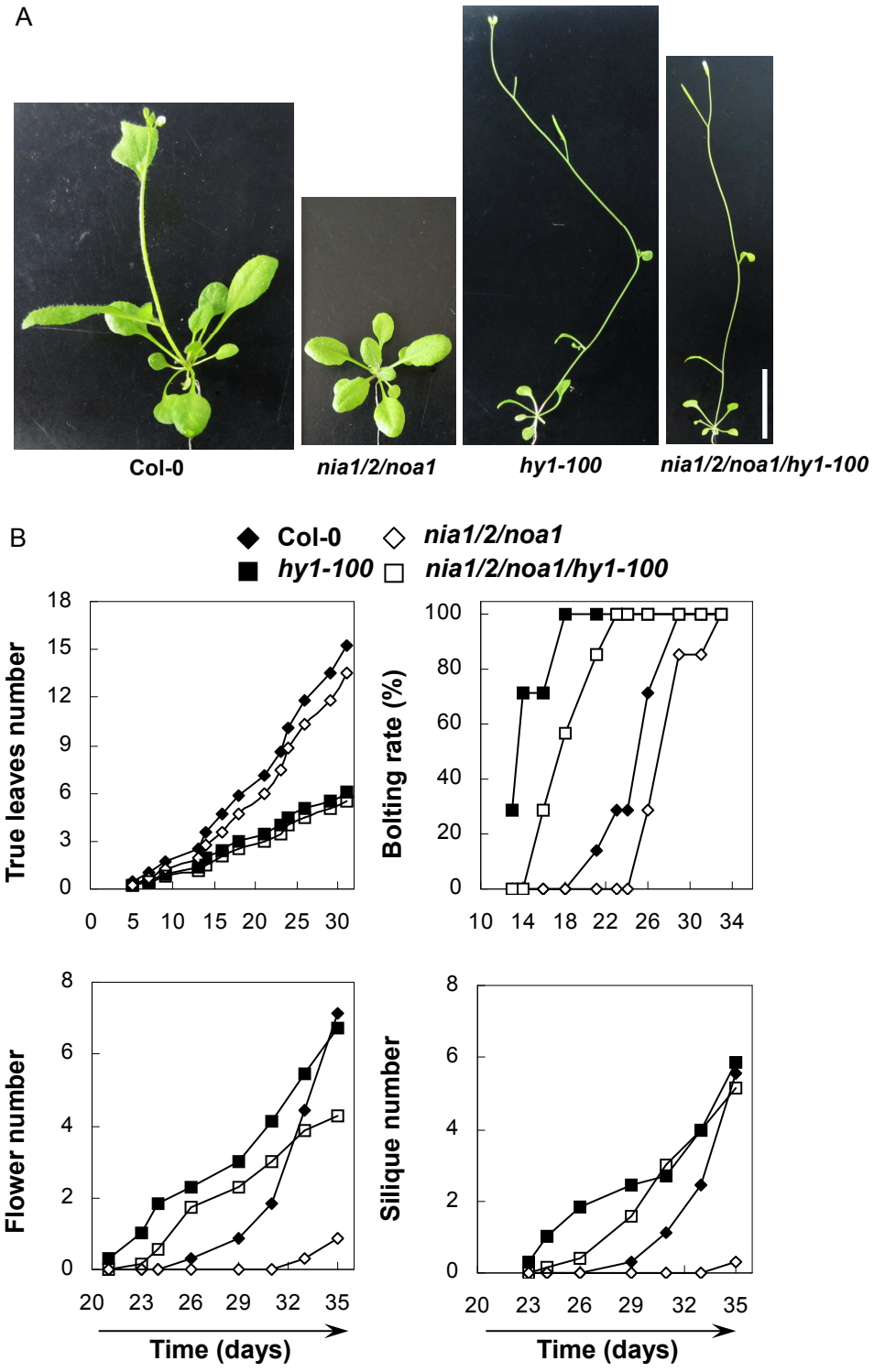

Figure S5. Developmental phenotypes of wild-type, *nia1/2/noa1*, *hy1-100*, and *nia1/2/noa1/hy1-100* plants at the vegetative and reproductive growth stages. Morphology

of indicated genotypes grown for 24 d in the soil under long-day conditions (A). Bar = 2 cm. Time course analysis of true leaves number, bolting rate, flower and silique numbers of corresponding genotypes (n = 30; B).

Figure S6

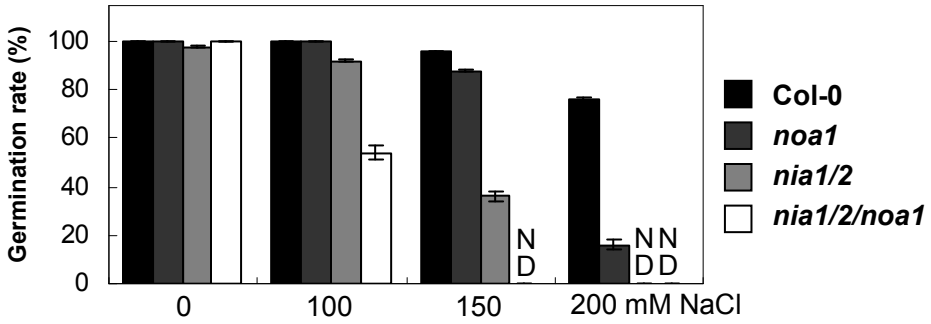

Figure S6. Dose-dependent inhibition of seed germination in wild-type (Col-0), *noa1*, *nia1/2*, and *nia1/2/noa1* induced by increasing NaCl concentrations for 8 d (n = 100). ND: none detected.

Figure S7

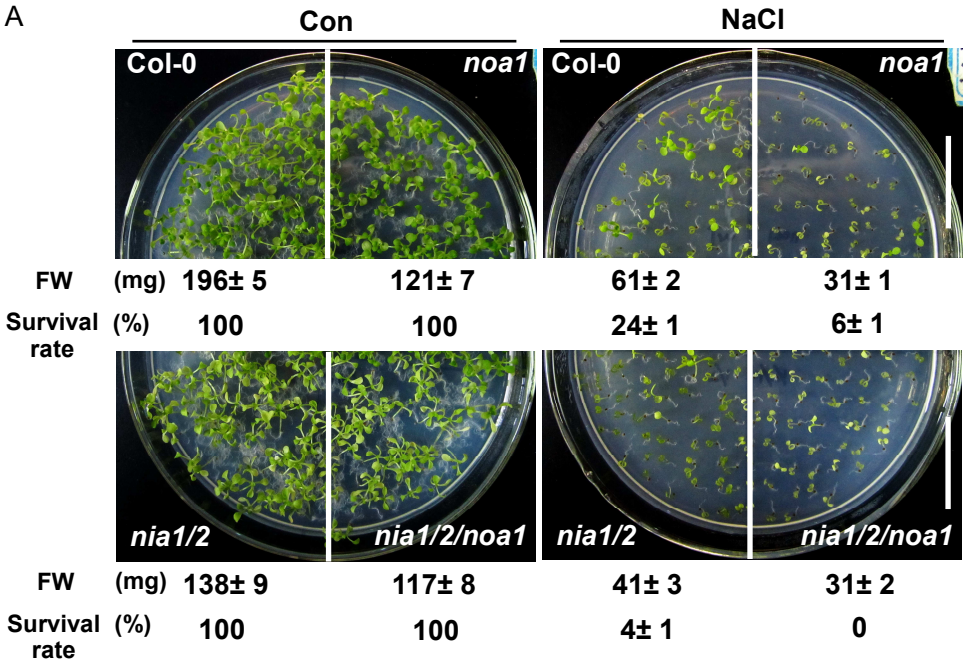

Figure S7. Fresh weight and survival rate of stratified seeds of wild-type (Col-0), *noa1*, *nia1/2*, and *nia1/2/noa1* with or without 150 mM NaCl treatment for 20 d. Bar = 2 cm.

Figure S8

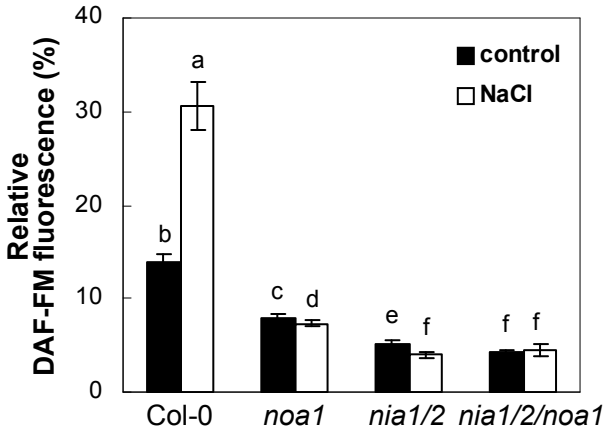

Figure S8. DAF-FM-associated fluorescence in seedling roots of Col-0, *noa1*, *nia1/2*, and *nia1/2/noa1*. 5-d-old seedlings of each ecotype were treated with or without 150 mM NaCl for 1 hr. Then, the DAF-FM fluorescence in seedling roots was detected by LSCM, and means of corresponding intensity were calculated by Leica software. Data are means ± SE from three independent experiments. Mean values were compared by a multiple comparison on the basis of the nested ANOVA, with genotypes nested within different treatments [model: phenotypic indicators = replication + treatment + sample (treatment)]. Mean values with different letters denote a significant difference according to multiple comparison (P<0.05).

Figure S9

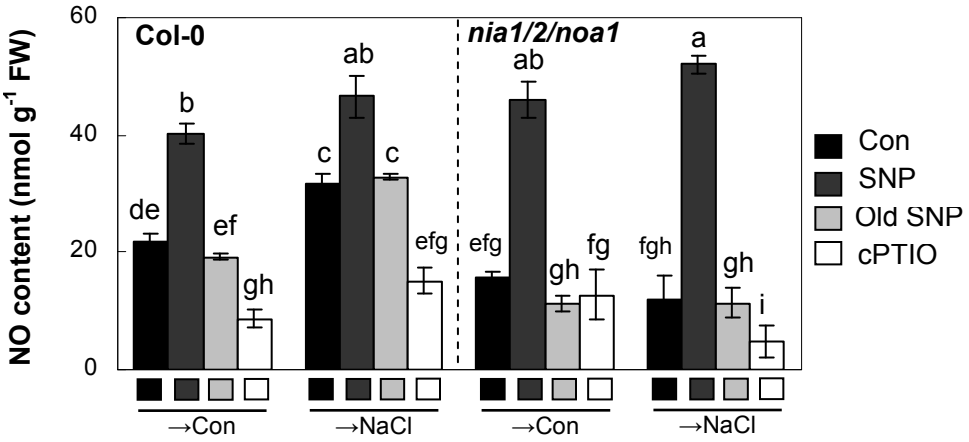

Figure. S9. Determination of NO content in Col-0 and *nia1/2/noa1* seedlings by Griess reagent assay. After pre-incubation in the absence or presence of 10 μM SNP, 10 μM Old SNP, or 400 μM cPTIO for 1 hr, 5-d-old seedlings of wild-type and *nia1/2/noa1* mutant were treated with or without 150 mM NaCl for another 1 hr. NO contents of seedlings were then detected by using Griess reagent. Samples pretreated with 1 mM cPTIO, the scavenger of NO for 30 min, were used as blanks. Data are means ± SE from three independent experiments. Mean values were compared by a multiple comparison on the basis of the nested ANOVA, with genotypes nested within different treatments [model: phenotypic indicators = replication + treatment + sample (treatment)]. Mean values with different letters denote a significant difference according to multiple comparison (P<0.05).

Figure S10

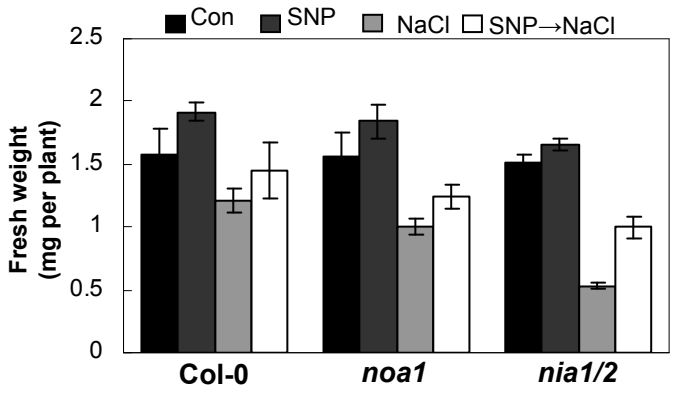

Figure S10. Effects of SNP on growth of Col-0, *noa1*, and *nia1/2* mutant plants. Plant seedlings were grown in the absence of NaCl or after 150 mM NaCl treatment for 7 d preceded by pretreatment for 5 d with or without 1.0 μM SNP. Corresponding fresh weight were then measured. Data are means ± SE from three independent experiments.

Figure S11

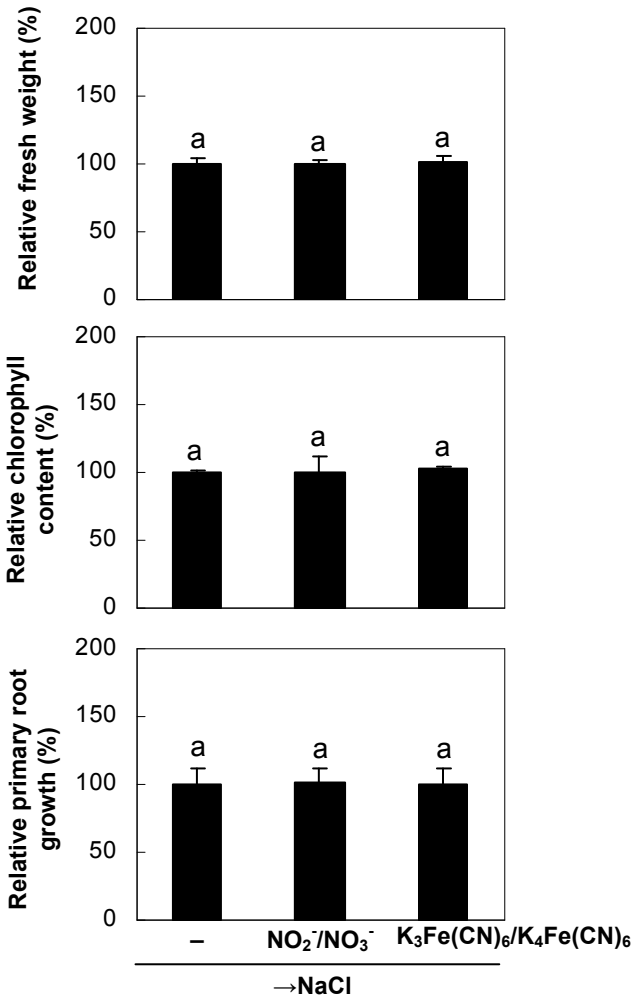

Figure S11. Effects of NO_2^-/NO_3^-, $K_3Fe(CN)_6/K_4Fe(CN)_6$ and NaCl on growth of Col-0 seedlings. Plants were exposed with 150 mM NaCl for 7 d preceded by pretreatment for 5 d with or without 1.0 μM/1.0 μM NO_2^-/NO_3^-, or 1.0 μM/1.0 μM $K_3Fe(CN)_6/K_4Fe(CN)_6$. Relative fresh weight, chlorophyll content, and primary root growth were then measured, taking 150 mM NaCl stressed alone samples as 100%. Data are means ± SE from three independent experiments. Bars with different letters are significantly different compared with NaCl stressed alone samples at the $P < 0.05$ level according to multiple comparison.

Figure S12

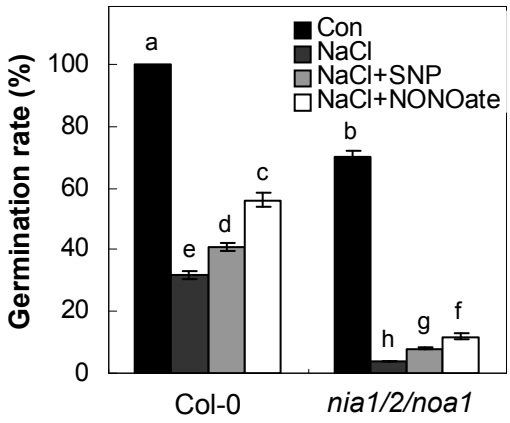

Figure S12. Germination rate of wild-type (Col-0) and *nia1/2/noa1* mutant seeds in the absence or presence of 150 mM NaCl with or without 1 μM SNP or 1 μM NONOate for 3 d. Data are means ± SE from three independent experiments with at least 100 seeds per experiment. Mean values were compared by a multiple comparison on the basis of the nested ANOVA, with genotypes nested within different treatments [model: phenotypic indicators = replication + treatment + sample (treatment)]. Mean values with different letters denote a significant difference according to multiple comparison (P<0.05).

Figure S13

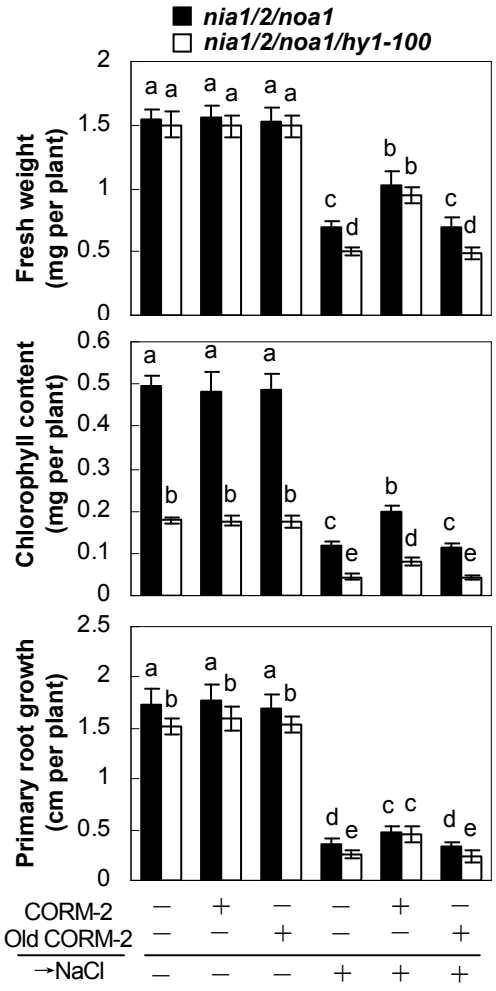

Figure S13. Effects of CORM-2 and Old CORM-2 on salt hypersensitivity phenotype of *nia1/2/noa1* and *nia1/2/noa1/hy1-100* mutant plants. Mutant plants were grown in the absence of NaCl or after 150 mM NaCl treatment for 7 d preceded by pretreatment for 5 d with or without 0.1 μM CORM-2 or 0.1 μM Old CORM-2. Corresponding fresh weight, chlorophyll content, and primary root growth were then measured. The Old CORM-2 were obtained as the negative controls. Data are means ± SE from three independent experiments. Mean values were compared by a multiple comparison on the basis of the nested ANOVA, with genotypes nested within different treatments [model: phenotypic indicators = replication + treatment + sample (treatment)]. Mean values with different letters denote a significant difference according to multiple comparison (P<0.05).

Figure S14

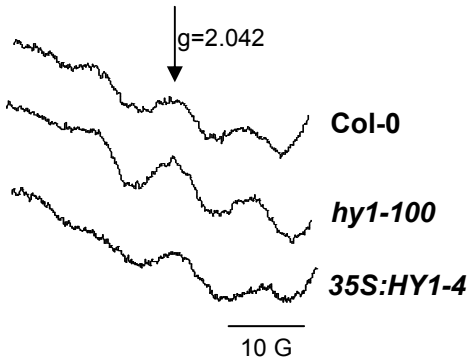

Figure. S14. Determination of NO content in Col-0, *hy1-100*, and *35S:HY1-4* seedlings by Electron Paramagnetic Resonance (EPR). 5-d-old seedlings of wild-type and mutants were treated with 150 mM NaCl for 1 hr, and then NO contents of seedlings were detected by EPR. The signals were recorded at the identical EPR settings.

Figure S15

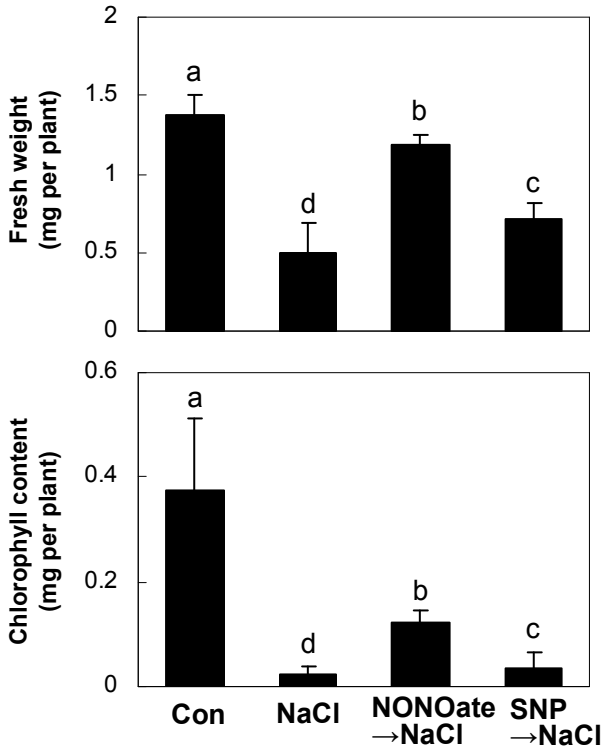

Figure S15. Effects of NONOate and SNP on growth and chlorophyll contents of *hy1* mutant plants. Mutant plants were grown in the absence of NaCl or after 150 mM NaCl treatment for 7 d preceded by pretreatment for 5 d with or without 1.0 μM NONOate or 1.0 μM SNP. Corresponding fresh weight and chlorophyll content were then measured. Data are means ± SE from three independent experiments. Mean values with different letters denote a significant difference according to multiple comparison (P<0.05).

Figure S16

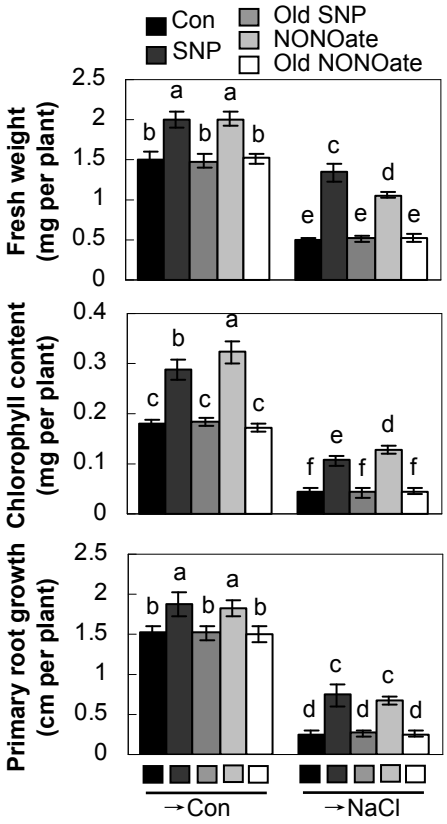

Figure S16. Effects of fresh and old NO-releasing compounds on salt hypersensitivity phenotype of *nia1/2/noa1/hy1-100* mutant plants. Mutant plants were grown in the absence of NaCl or after 150 mM NaCl treatment for 7 d preceded by pretreatment for 5 d with or without 1.0 μM SNP, 1.0 μM Old SNP, 1.0 μM NONOate, or 1.0 μM Old NONOate. Corresponding fresh weight, chlorophyll content, and primary root growth were then measured. The Old SNP/Old NONOate was obtained as the negative controls. Data are means ± SE from three independent experiments. Mean values with different letters denote a significant difference according to multiple comparison (P<0.05).

Supplementary Table 1. The sequences of PCR primers for genotyping.

Primer name	Sequences (5'→3')
NOA1-LP	GCACCTACACCACAGGCAAGC
NOA1-RP	CCAATTGGCAATGTTGGTCG
NIA1-LP	TACGACGACTCCTCAAGCGAC
NIA1-RP	GGCTATAGATCCCGCATCGAC
NIA2-LP	ACGGCGTGGTTCGTTCTTACA
NIA2-RP	ACCTTCTTCGTCGGCGAGTTC
LBb1.3	ATTTTGCCGATTTCGGAAC

Supplementary Table 2. The sequences of PCR primers for real-time RT-PCR.

Locus	Primer name	Sequences (5'→3')
At2g26670	*HY1-F*	CGTCCTGTTGCTAAATG
	HY1-R	TTCCAGCCCCGTGTTCT
At1g69720	*HO-3-F*	CCACTTTCCAGCGAGCACA
	HO-3-R	ATAGCCGCCGCAGTCAC
At1g58300	*HO-4-F*	TCTTGCCGCTTTCCTGC
	HO-4-R	GCTGCTGCCACAACATTC
At5g52310	*RD29A-F*	ATCACTTGGCTCCACTGTTGTTC
	RD29A-R	ACAAAACACACATAAACATCCAAAGT
At5g66400	*RAB18-F*	AAGAAGAACATGGCGTCTTACCA
	RAB18-R	TGCTGCTGGATCGGGTTT
At1g27730	*ZAT10-F*	AGGCTCTTACATCACCAAGATTAG
	ZAT10-R	TACACTTGTAGCTCAACTTCTCCA
At5g59820	*ZAT12-F*	TGTCCCATATGTGGAGTGGA
	ZAT12-R	ATTGTCCACCATCCCTAGACT
At2g43350	*GPX3-F*	CATCACCTCTTGAAATTGAGAAGGA
	GPX3-R	CAGACATGTTTGATGCGATGC
At1g07890	*cAPX1-F*	ACTCTGGGACGATGCCACAAG
	cAPX1-R	TCTCGACCAAAGGACGGAAAA
At3g09640	*cAPX2-F*	TGGTCGGATGGGACTCAAT
	cAPX2-R	AAGAGCCTTGTCGGTTGGT
At4g25100	*FSD1-F*	GCTCGGCTCTTTCCCATTGC
	FSD1-R	CAGCTTCCCAAGACACAAGATTGG
At3g18780	*actin2/7-F*	TCGTTTCGCTTTCCTTAG
	actin2/7-R	CTTCACCATTCCAGTTCC

Supplementary Table 3. Comparative analysis of seedling growth inhibition of wild-type, *hy1-100*, *nia1/2/noa1*, and *nia1/2/noa1/hy1-100* mutants grown upon 150 mM NaCl stress for 7 d. Data are means ± SE from three independent experiments. Different letters within columns indicate significant differences at the $P < 0.05$ level according to multiple comparison.

Genotype	Primary root growth (cm per plant)	Fresh weight (mg per plant)
Col-0	0.85 ± 0.13^{a}	1.29 ± 0.14^{a}
hy1-100	0.46 ± 0.09^{b}	1.07 ± 0.07^{b}
nia1/2/noa1	0.35 ± 0.07^{c}	0.70 ± 0.04^{c}
nia1/2/noa1/hy1-100	0.26 ± 0.04^{d}	0.50 ± 0.04^{d}

Methods for supplementary data

Chemicals

NO_2^-/NO_3^- and $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ were used as the negative controls of SNP treatment (Ederli et al., 2006; Shi et al., 2007; Xie et al., 2008). The concentrations of above compounds used in this study were determined in pilot experiments from which maximal induced responses were obtained.

Survival rate analysis

After germination, survival rates of Arabidopsis seedlings were measured according to the method described by Zhao et al. (2007).

NO detection by Griess reagent

NO production was determined using the Griess reagent as described (Xie et al., 2008; Xuan et al., 2012). Meanwhile, samples of various treatments, which were preincubated with 1 mM cPTIO for 30 min, were regarded as the blank to avoid the interference of nitrite. Absorbance was assayed at 540 nm, NO content was calculated by comparison to a standard curve of $NaNO_2$.

Western-blot analysis for HY1

Arabidopsis seedlings were collected for western-blot analysis of HY1. The primary antibody used was rabbit polyclonal antibody raised against the recombinant mature HY1 protein with a molecular mass of 26.6 kDa (Xie et al., 2011). Immune complexes were detected using horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG. The color was developed with a solution containing DAB as the HRP substrate.

References for Supplementary data

Ederli L, Morettini R, Borgogni A, Wasternack C, Miersch O, Reale L, Ferranti F, Tosti N, Pasqualini S. 2006. Interaction between nitric oxide and ethylene in the induction of alternative oxidase in ozone-treated tobacco plants. *Plant Physiology* **142,** 595–608.

Shi Q, Ding F, Wang X, Wei M. 2007. Exogenous nitric oxide protect cucumber roots against oxidative stress induced by salt stress. *Plant Physiology and Biochemistry* **45,** 542–550.

Xie YJ, Ling TF, Han Y, Liu KL, Zheng QS, Huang LQ, Yuan XX, He ZY, Hu B, Fang L, Shen ZG, Yang Q, Shen WB. 2008. Carbon monoxide enhances salt tolerance by nitric oxide-mediated maintenance of ion homeostasis and up-regulation of antioxidant defence in wheat seedling roots. *Plant, Cell and Environment* **31,** 1864–1881.

Xie YJ, Xu S, Han B, Wu MZ, Yuan XX, Han Y, Gu Q, Xu DK, Yang Q, Shen WB. 2011. Evidence of Arabidopsis salt acclimation induced by up-regulation of *HY1* and the regulatory role of RbohD-derived reactive oxygen species synthesis. *The Plant Journal* **66,** 280–292.

Xuan W, Xu S, Li M, Han B, Zhang B, Zhang J, Lin Y, Huang J, Shen W, Cui J. 2012. Nitric oxide is involved in hemin-induced cucumber adventitious rooting process. *Journal of Plant Physiology* **169,** 1032–1039.

Zhao MG, Tian QY, Zhang WH. 2007. Nitric oxide synthase-dependent nitric oxide production is associated with salt tolerance in Arabidopsis. *Plant Physiology* **144,** 206–217.