

Supporting Information

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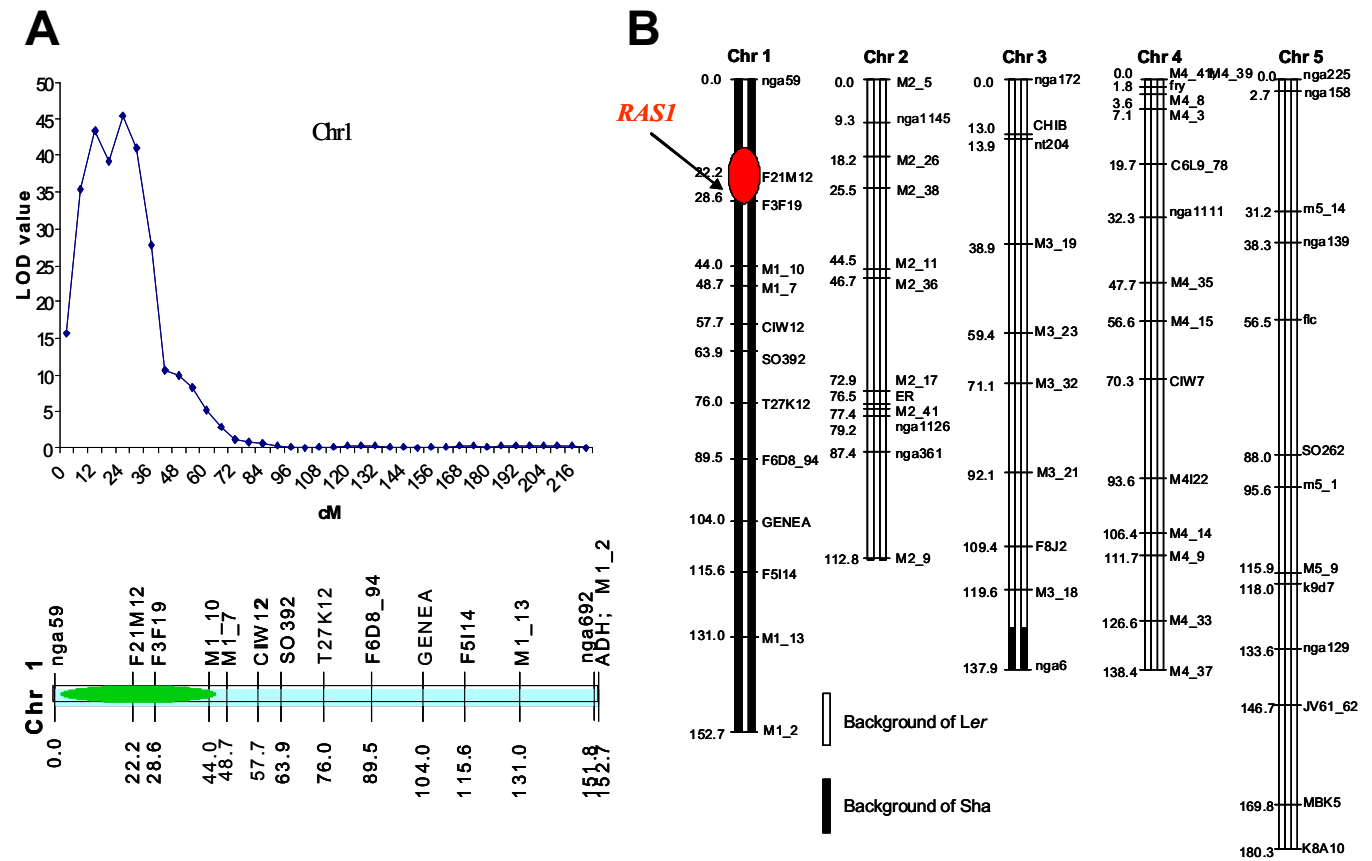


Fig. S1. (A) Major QTL for salt tolerance in the Bay-0 × Sha RIL population colocalizes with the *RAS1* QTL. Salt tolerance, measured as the percentage of GS on MS medium supplemented with 120 mM NaCl, was used to map QTLs. (B) The genetic background of CS24560, which was used to develop the fine mapping population, is shown.

A

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col : MPNTSSSQSFTIFVDGWLIRHRYFVBEQLMCASSLDETNRISLBEQQSLVAQFLSHCLQYY : 60
Ler : MPNTSSSQSFTIFVDGWLIRHRYFVBEQLMCASSLDETNRISLBEQQSLVAQFLSHCLQYY : 60
Sha : MPNTSSSQSFTIFVDGWLIRHRYFVBEQLMCASSLDETNRISLBEQQSLVAQFLSHCLQYY : 60

col : QEKFAVS LAGDNVTFEFCPPWFNSYAKLLWVGDFKPSLVFKLTEVSVADLTRHQKDR : 120
Ler : QEKFAVS LAGDNVTFEFCPPWFNSYAKLLWVGDFKPSLVFKLTEVSVADLTRHQKDR : 120
Sha : QEKFAVS LAGDNVTFEFCPPWFNSYAKLLWVGDFKPSLVFKLTEVSVADLTRHQKDR : 120

col : SSLKSETRREREVMRDFALVQSSVADPPVMLAARRVGAVGMVDGEBTDLEAMEV LKAG : 180
Ler : SSLKSETRREREVMRDFALVQSSVADPPVMLAARRVGAVGMVDGEBTDLEAMEV LKAG : 180
Sha : SSLKSETRREREVMRDFALVQSSVADPPVMLAARRVGAVGMVDGEBTDLEAMEV LKAG : 180

col : MAAAMNADQLRCSTVGKVV EILT PPOATKVLRTIGQLHLRLRRDRQERA : 230
Ler : MAAAMNADQLRCSTVGKVV EILT PPOATKVLRTIGQLHLRLRRDRQERA : 230
Sha : MAAAMNADQLRCSTVGKVV EILT PPOAT----- : 209

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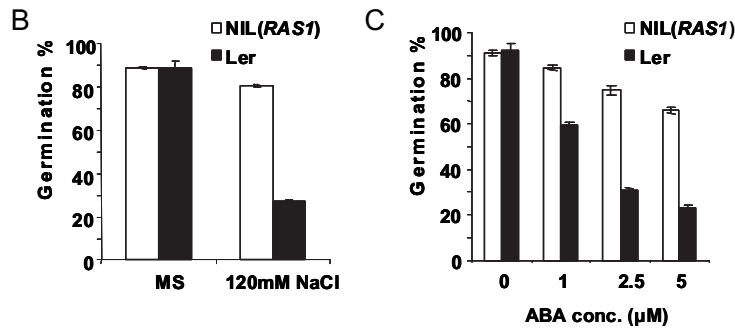


Fig. S2. RAS1 sequence polymorphism and the effect of RAS1 locus on salt and ABA sensitivity. (A) Amino acid sequence alignment of RAS1 from Ler, Col-0, and Sha. Germination rate of Ler and NIL(RAS1) on MS with 120 mM NaCl (B) or various concentrations (conc.) of ABA (C). Values are mean \pm SE of three replicates, with about 90 seeds per replicate.

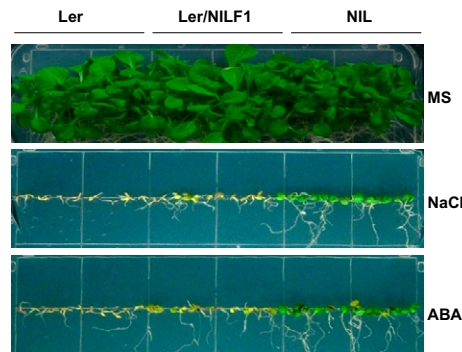


Fig. S3. RAS1 allele of Ler is dominant over the Sha allele. Shown is the phenotype of F1 seedlings from a cross between Ler and NIL and their parents grown on MS or MS supplemented with 120 mM NaCl or 2.5 μ M ABA.

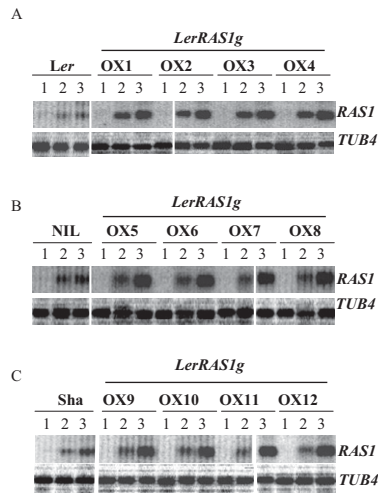


Fig. 54. *RAS1* gene expression in transgenic complementation lines. *RAS1* expression in transgenic Ler (*A*), NIL(*RAS1*) (*B*), and Sha (*C*) lines expressing *Ler RAS1* gene with its own promoter (*LerRAS1g*) and WT in response to ABA and NaCl. 1, control before treatment; 2, 100 μ M ABA treatment for 0.5 h; 3, 150 mM NaCl treatment for 1 h; OX, overexpression line. *TUB4* expression serves as the RNA loading control.

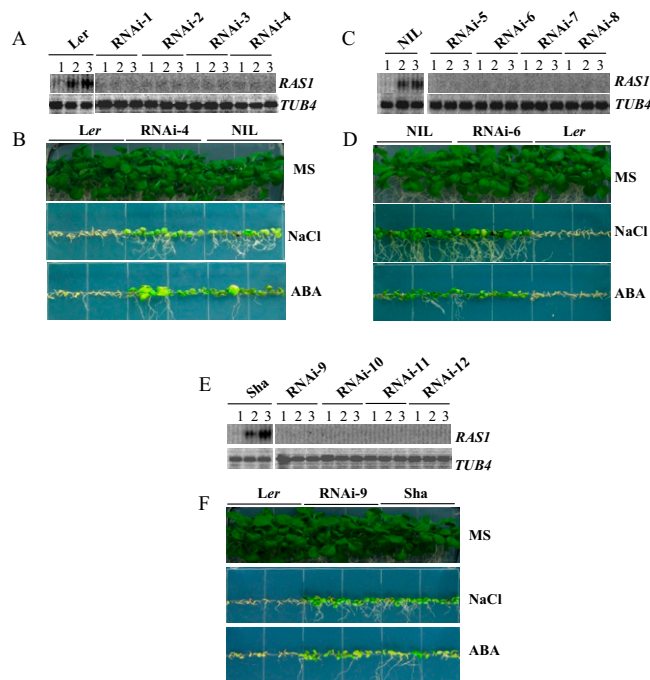


Fig. 55. Germination and early seedling growth of *RAS1* RNAi knockdown lines and their respective WT on MS medium supplemented with ABA and NaCl. *RAS1* expression under ABA and NaCl treatments is shown for *RAS1*-RNAi transgenic lines in Ler (*A*), NIL(*RAS1*) (*C*), and Sha (*E*) genotypes. 1, control before treatment; 2, 100 μ M ABA treatment for 0.5 h; 3, 150 mM NaCl treatment for 1 h. *TUB4* expression serves as the RNA loading control. ABA and NaCl stress response phenotypes are shown for the Ler (*B*), NIL(*RAS1*) (*D*), and Sha (*F*) RNAi lines. Seeds were sown on medium containing NaCl (120 mM) or ABA (2.5 μ M) and allowed to grow. Expression of *RAS1* was examined in four RNAi lines for each genetic background. Salt and ABA responses of a representative RNAi line for each genetic background are shown.

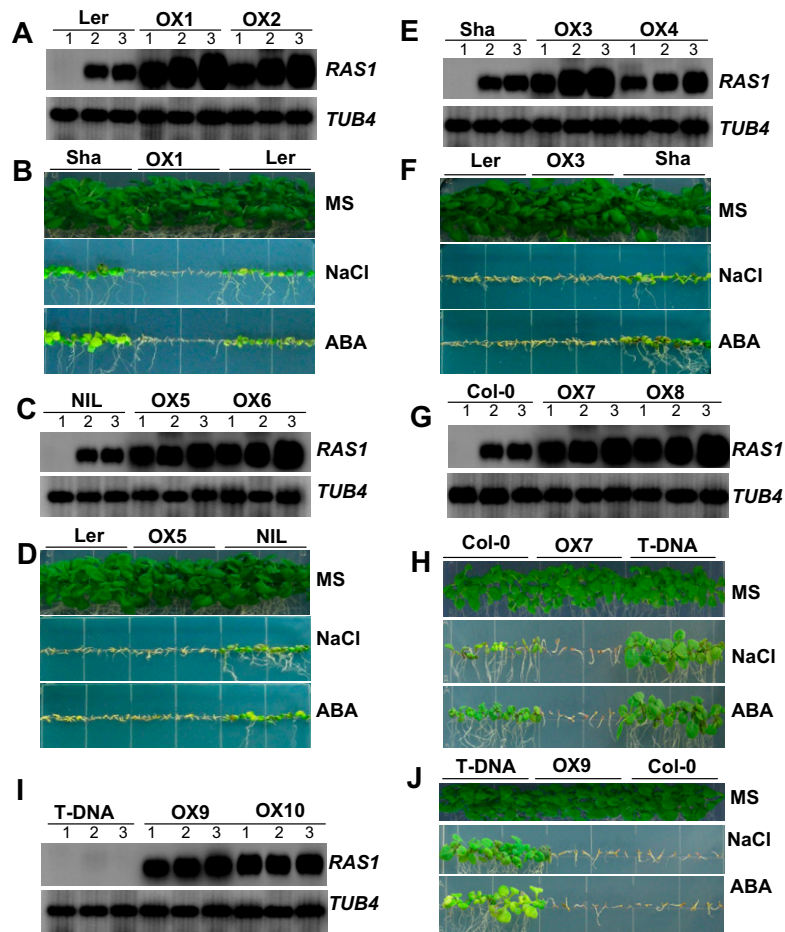


Fig. 56. ABA and NaCl sensitivity of *Ler-RAS1* overexpressing transgenic lines (OX). *RAS1* expression in transgenic *Ler* (A), *NIL(RAS1)* (C), *Sha* (E), *Col-0* (G), and T-DNA line (Salk_058470) (I) and their respective WT plants. 1, control; 2, 100 μ M ABA for 0.5 h; 3, 150 mM NaCl for 1 h. *TUB4* expression serves as the RNA loading control. Germination and seedling growth of *Ler RAS1* overexpressing transgenic *Ler* (B), *NIL(RAS1)* (D), *Sha* (F), *Col-0* (H), and T-DNA line (Salk_058470) (J) under ABA and NaCl treatment. Seedlings were grown on MS, MS with 80 mM NaCl, or 1 μ M ABA in B and H, whereas in the rest of the cases, seedlings were grown on 120 mM NaCl or 2.5 μ M ABA.

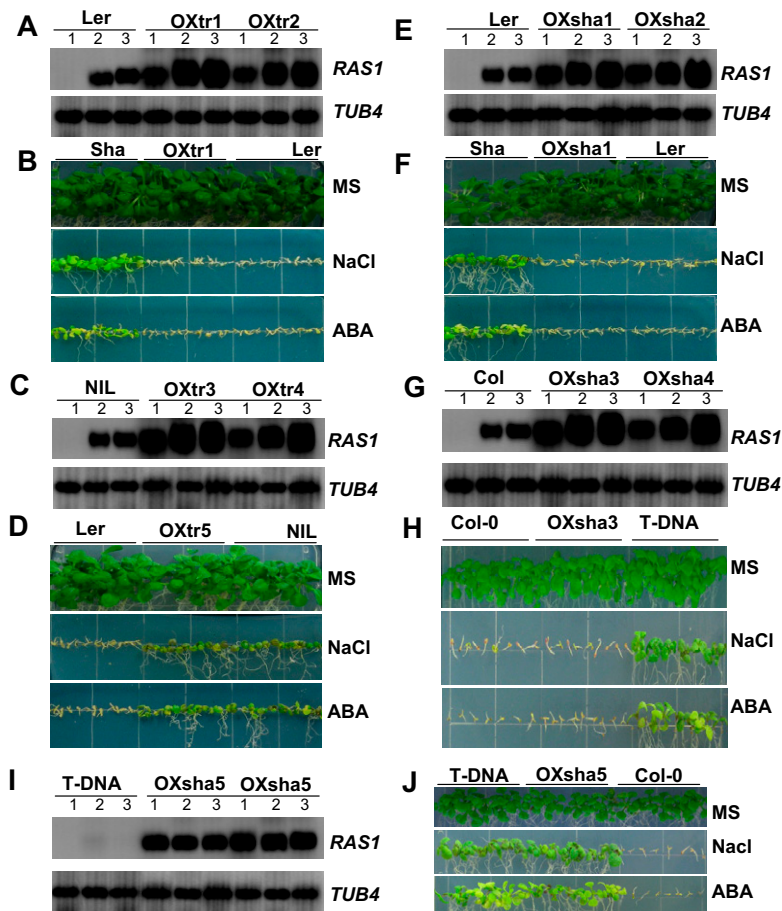


Fig. S7. Effect of overexpression of C-terminally truncated *RAS1* on salt and ABA responses. Northern blot analysis of *RAS1* expression in transgenic Ler (A) and NIL(*RAS1*) (C) overexpressing the C-terminally truncated Ler-*RAS1* (OXtr) and transgenic Ler (E), Col-0 (G), and T-DNA line (Salk_058470) (I) overexpressing the Sha-(OXsha). 1, control; 2, 100 μ M ABA for 0.5 h; 3, 150 mM NaCl for 1 h. *TUB4* expression serves as RNA loading control. Effect of ABA and NaCl on germination and seedling growth of transgenic lines overexpressing C-terminally truncated Ler *RAS1* [B, Ler; D, NIL(*RAS1*)] or Sha *RAS1* (F, Ler; H, Col-0; J, Salk_058470). Seedlings were grown on MS, MS with 120 mM NaCl, or 2.5 μ M ABA.

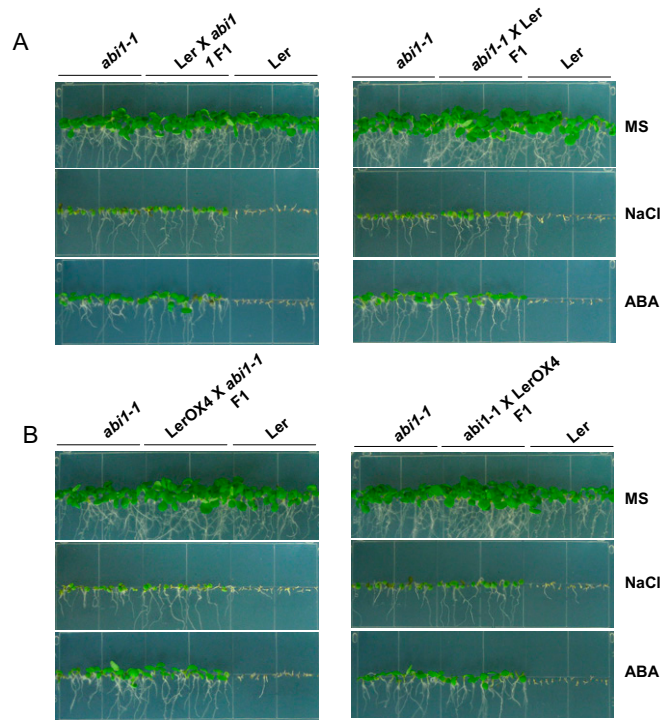


Fig. S8. The *abi1-1* mutation suppresses ABA and salt hypersensitivity conferred by *RAS1* overexpression. (A) F1 from the reciprocal crosses between *abi1-1* and Ler shows resistance to ABA and NaCl during germination and early seedling growth. (B) F1 from the reciprocal crosses between *abi1-1* and the Ler-*RAS1*-overexpressing transgenic line (Ler background, line OX4) shows resistance to ABA and NaCl during germination and early seedling growth.



Fig. S9. Response of different *Arabidopsis* accessions to salt stress. Seeds were germinated on MS medium supplemented with 120 mM NaCl and were grown for 20 days.

Table S1. Putative QTLs for salt tolerance in the RIL population derived from Ler and Sha

Trait	QTL*	Chr	Markers bordering QTL	Peak LOD	a [†]	PVE [‡]	R ^{2§}	DPE [¶]
GS	qGS1	1	nga59-M1_7	20.3	0.35	76.6	49.6	S
RL	qRL1	1	nga59-F21M12	4.12	1.42	19.9	15.7	S
	qRL2	1	M1_2-ADH	2.56	0.94	9.9	9.8	S
	qRL3	3	M3_19-M3_23	2.74	0.96	10.3	10.7	S
	qRL4	4	M4_35-M4_15	2.10	-0.87	8.5	7.9	L

*QTLs are named by abbreviation plus a number; GS, % of GSs under 120 mM NaCl treatment; RL, RL under 120 mM NaCl treatment.

[†]Additive effect on the Sha allele.

[‡]Percentage of total PVE explained by the QTL.

[§]R² values are calculated based on ANOVA of the trait values explained by individual markers linked to different QTLs.

[¶]Direction of phenotypic effect; L and S indicate Ler and Sha, respectively.