Intracellular calcium transients during plant–pathogen interactions are necessary early events leading to local and systemic acquired resistance\(^6\). Salicylic acid, a critical messenger, is also required for both of these responses\(^2\), but whether and how salicylic acid level is regulated by \(Ca^{2+}\) signalling during plant–pathogen interaction is unclear. Here we report a mechanism connecting \(Ca^{2+}\) signal to salicylic-acid-mediated immune response through calmodulin, AtSR1 (also known as CAMTA3), a \(Ca^{2+}\)/calmodulin-binding transcription factor, and EDS1, an established regulator of salicylic acid level. Constitutive disease resistance and elevated levels of salicylic acid in loss-of-function alleles of \textit{Arabidopsis} \textit{AtSR1} suggest that \textit{AtSR1} is a negative regulator of plant immunity. This was confirmed by epistasis analysis with mutants of compromised salicylic acid accumulation and disease resistance. We show that \textit{AtSR1} interacts with the promoter of \textit{EDS1} and represses its expression. Furthermore, \(Ca^{2+}\)/calmodulin-binding to \textit{AtSR1} is required for suppression of plant defence, indicating a direct role for \(Ca^{2+}\)/calmodulin in regulating the function of \textit{AtSR1}. These results reveal a previously unknown regulatory mechanism linking \(Ca^{2+}\) signalling to salicylic acid level.

\textit{Arabidopsis thaliana} signal responsive (\textit{AtSR}) proteins belong to a class of \(Ca^{2+}\)/calmodulin (\textit{CaM})-binding transcription factors\(^2\). In animals, \textit{AtSR} homologues are involved in diverse functions\(^8,9\). Although \textit{AtSR} proteins are implicated in plant responses to stresses\(^8\), the specific function of \textit{AtSR}s remains unknown. To determine the function of \textit{AtSR1}, we isolated two loss-of-function mutants (\textit{Atsr1-1} and \textit{Atsr1-2}) in \textit{Arabidopsis} (Supplementary Figs 1a and 2a, e). At 25–27 \(^\circ\)C (12 h or other photoperiods), no noticeable difference between the wild-type and \textit{Atsr1} mutants was observed (Fig. 1a). However, \textit{Atsr1-1} showed elevated resistance to virulent \textit{Pseudomonas syringae pv. tomato} DC3000 (hereafter called \textit{Pst} DC3000; Fig. 1b), as well as avirulent \textit{Pst} (AvrRpt2; data not shown). Because elevated resistance to pathogens is usually correlated with induced expression of \textit{PR} genes\(^9\), we analysed the expression of \textit{PR1} in wild-type and \textit{Atsr1-1} plants inoculated with \textit{Pst} DC3000. In wild-type plants \textit{PR1} expression did not start until 24 h after inoculation, whereas in \textit{Atsr1-1} mutants its expression started 6 h after inoculation. However, the maximum expression of \textit{PR1} remained similar between wild-type and \textit{Atsr1} plants (Fig. 1c). The elevated disease resistance and sensitized \textit{PR1} expression in \textit{Atsr1-1} plants indicate that it is a repressor of plant immunity.

At 19–21 \(^\circ\)C (12-h photoperiod), \textit{Atsr1-1} plants showed reduced growth (Fig. 1a, d and Supplementary Fig. 2d). The expression of systemic acquired resistance-associated marker genes\(^10\), \textit{PR1}, \textit{PR2} and \textit{PR5}, was constitutively activated under lower temperature in \textit{Atsr1-1} plants (Fig. 1f). Predictably, the disease resistance of \textit{Atsr1-1} plants was also enhanced in comparison with plants grown under higher temperature (Fig. 1b, e). The \textit{Atsr1} plants displayed chlorosis and autonomous lesions (Fig. 1g and Supplementary Figs 2b, d). Plants undergoing hypersensitive response produce reactive oxygen species (ROS) and autofluorescent compounds\(^11,12\). Staining for \(H_2O_2\) revealed numerous brown patches on \textit{Atsr1-1} leaves, which were comparable to wild-type plants infected with incompatible \textit{Pst} (AvrRpt2) (Fig. 1h and Supplementary Fig. 3). \textit{Atsr1-1} leaves also showed extensive autofluorescence (Fig. 1i). These results indicate that \textit{Atsr1} plants grown at a lower temperature show hallmarks of constitutive defence responses commonly found in lesion-mimicking mutants\(^11\) or wild-type plants inoculated with avirulent bacterial pathogens\(^12\). The temperature-dependent autoimmunity further suggests that \textit{AtSR1} represses R-protein-mediated defence activation. Recent reports show that the stability of active R proteins is regulated by co-chaperone RAR1 in plants in a temperature-dependent manner, with lower temperatures favouring the accumulation of R proteins\(^13,14\).

Conceivably, lower temperatures might favour the accumulation of some R proteins in \textit{Arabidopsis}, but \textit{AtSR1} represses the mis-activation of defence whereas its absence in \textit{Atsr1} does not. The expression of \textit{AtSR1} cDNA in \textit{Atsr1} mutants restored all mutant phenotypes (Supplementary Fig. 4), confirming that the \textit{Atsr1} phenotypes are caused by loss of \textit{AtSR1}.

Because \textit{Atsr1} mutants resemble mutants with increased salicylic acid levels\(^11,15\), we quantified salicylic acid in the mutant and wild-type plants grown at 19–21 \(^\circ\)C. Free and conjugated salicylic acid levels were increased ~7- and ~8-fold, respectively, in the \textit{Atsr1} plants (Fig. 2a, b). In uninfected plants grown at 25–27 \(^\circ\)C, salicylic acid levels in \textit{Atsr1} and wild-type plants were similar. However, the salicylic acid level increased faster in \textit{Atsr1} than in wild-type plants when inoculated with \textit{Pst} DC3000 (Supplementary Fig. 5). Previous studies have shown that elevating salicylic acid levels alone is enough to cause an enhanced immune response and reduced growth\(^9,15\). Expressing the salicylic-acid-degrading enzyme NahG suppressed both disease resistance and retarded growth in some disease-resistant mutants (\textit{acd6}, \textit{bon1} and \textit{ssi1}) with elevated salicylic acid levels\(^9,17\), but only disease resistance in other mutants (\textit{mpk4} and \textit{ndu1})\(^18,19\). To determine if the reduced growth and enhanced disease resistance of \textit{Atsr1} mutants are caused by elevated salicylic acid levels or other mechanism(s), we eliminated salicylic acid by expressing \textit{NahG} in wild-type and \textit{Atsr1-1} plants. Wild-type and \textit{Atsr1-1} plants expressing \textit{NahG} appeared to be similar but both were bigger than the wild type (Fig. 2c-e). Furthermore, constitutively activated \textit{PR1} expression was blocked in \textit{Atsr1-1} \textit{NahG} plants (Fig. 2c); both \textit{Atsr1-1} \textit{NahG} and wild-type \textit{NahG} plants were more sensitive to \textit{Pst} DC3000 than wild-type plants (Fig. 2f). These results indicate that the elevated salicylic acid level is the major cause of \textit{Atsr1-1} phenotypes.

Consistent with elevated salicylic acid levels, the expression of \textit{ICS1}, \textit{PAD4}, \textit{EDS1} and \textit{EDSS}, important positive regulators of salicylic acid biosynthesis, is highly induced in \textit{Atsr1} plants.

\(^1\)Center for Integrated Biotechnology and Department of Horticulture, \(^2\)Department of Chemistry, Washington State University, Pullman, Washington 99164-6414, USA. \(^3\)Department of Biology and Program in Molecular Plant Biology, Colorado State University, Fort Collins, Colorado 80523-1878, USA. \(^4\)Present address: Bioanalytical Services, Primera Analytical Solutions Corp., 259 Wall Street, Princeton, New Jersey 08540, USA.

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plants grown at indicated temperature. Therefore, the elevated expression of these genes in the ~EDS1~ mutant shows sensitized defence responses. 

**Figure 1** The Atsr1-I mutant shows sensitized defence responses. 

- **a**, Phenotypic comparison. Thirty-two-day-old (left) and thirty-five-day-old (right) plants grown at indicated temperature. 
- **b**, Disease resistance test. *Pst* DC3000 (optical density at 600 nm (OD600) of 0.001) was infiltrated into rosette leaves (4 weeks, 25–27 ºC), and the colony-forming units (c.f.u.) at 3 days post inoculation (d.p.i.) are shown. 
- **c**, Time course of PR1 expression. Rosette leaves (4-week-old plants, 25–27 ºC) were infiltrated with *Pst* DC3000 (OD600 0.001) and samples were taken at the indicated time for northern blot analysis. 
- **d**, Growth comparison in terms of fresh rosette weight (5 weeks, 19–21 ºC). 
- **e**, Disease resistance test. *Pst* DC3000 (OD600 0.0001) was infiltrated into rosette leaves (19–21 ºC) and the c.f.u. at 3 d.p.i. are shown. 
- **f**, Constitutive induction of PR genes in the Atsr1-I mutant. Total RNA samples were prepared from rosette leaves grown at 19–21 ºC, identical blots were hybridized to indicated probes. 
- **g**, Early chlorosis of Atsr1-I leaf compared with wild-type leaf of the same age. 
- **h**, Production of 

*Arabidopsis* ecotype Columbia, was not used for epistasis analysis. The pad4 and ics1 single mutants, as well as the Atsr1 pad4 and Atsr1 ics1 double mutants, were more sensitive to *Pst* DC3000 than the wild type (Supplementary Fig. 6c). Furthermore, in the double mutants, constitutive expression of PR1 and the dwarf phenotype of Atsr1-1 are restored to wild-type levels (Supplementary Fig. 6d, e). 

The eds5 mutation also blocked the Atsr1 phenotypes (data not shown). These results suggest that AtSR1 functions at a step no later than PAD4 in the salicylic acid activation cascade. 

AtSR1 and its homologues bind to the conserved CGCG box and regulate the expression of target genes. Analysis of *ICS1* and *PR2* promoters revealed a typical CGCG box (ACGCGT) only in the *EDS1* promoter (–746 to –741, Supplementary Fig. 7a), indicating a direct regulation of *EDS1* by AtSR1. We showed that the AtSR1 DNA-binding domain (1–153 amino acids) binds the *EDS1* promoter fragment (–762 to –731) in an ACGCGT-dependent (Fig. 3a) and Ca2+/CalM-independent manner (data not shown). Chromatin immunoprecipitation assay further confirmed that a full-length AtSR1-YFP interacts with the *EDS1* promoter in vivo (Fig. 3a). To study the functional significance of AtSR1-binding to the *EDS1* promoter, the ~1.5-kb promoter of *EDS1* (*EDS1P*) was cloned and the ACGCGT element was mutated to ACCCGGT (*eds1p*) to abolish its interaction with AtSR1. Both *EDS1P* and eds1p were fused to luciferase (luc) and expressed in protoplasts of...
The activity of *EDS1::luc* is ~2-fold higher in *Atsr1* plants in comparison with wild-type plants (Fig. 3b), noticeably less than the four- to fivefold difference revealed by northern blot analysis (Supplementary Fig. 6a). In addition to the diluted feedback induction of *EDS1* by salicylic acid or other messengers in protoplast maintaining buffer, the introduction of an extra copy (or copies) of *EDS1* promoter into *Atsr1* mutants may have reduced the induction of *EDS1::luc* as well as endogenous *EDS1*, because the elevated expression of *EDS1* in *Atsr1* mutants is driven by unidentified positive transcription factor(s) (see model, Supplementary Fig. 7c). To test this, we generated stable wild-type and *Atsr1* transformants carrying pDL326 or pDL327. All wild-type plants carrying pDL326 or pDL327 grew like wild-type plants without these plasmids (data not shown); all the *Atsr1-1* plants carrying pDL327 (M327) grew like *Atsr1* plants without the plasmid (Fig. 3c and Supplementary Fig. 8b). Notably, most of the *Atsr1-1* plants carrying pDL326 (M326) showed varying degrees of phenotypic rescue (Supplementary Fig. 8a). Nearly 10% of them grew like wild-type plants during their entire life cycle (Fig. 3c and Supplementary Fig. 8a) and lacked *Atsr1* (Fig. 3d). Remarkably, the expression of endogenous *EDS1* in these lines was restored to wild-type levels (Fig. 3e and Supplementary Fig. 8c), indicating that the phenotypic restoration is due to the quenched *EDS1* expression. Consistently, constitutive *PR1* expression was also abolished in rescued M326 lines (Fig. 3e and Supplementary Fig. 8c). Segregation analysis of T2 progeny of M326 lines indicated that the phenotypic rescue is mostly correlated with particular insertion events rather than a dosage effect of insertion (data not shown). It seems that insertion of *EDS1* promoter at some particular positions in the *Atsr1* genome competes for the AC CGGT-binding positive regulator(s) and quenches the endogenous *EDS1* expression, although the precise mechanism remains to be resolved. Failure of *eds1p::luc* to rescue the *Atsr1* phenotype (Fig. 3c, e and Supplementary Fig. 8b) further supports this notion.

Functional tests of mutations in a null mutant background provide an effective strategy to study regulation of *EDS1* function by Ca²⁺/CaM. Three mutations (M1, I909V; M2, K907E; M3, A900–922) in the calmodulin-binding domain (CaMBD) of *Atsr1* (refs 7–9) were generated (Supplementary Fig. 1b). Wild-type *Atsr1* and *Atsr1(I909V)* bound CaM, whereas *Atsr1(K907E)* and *Atsr1(A900–922)* did not (Fig. 4a). Wild-type and mutated *Atsr1* plants were fused to Tag flag (Supplementary Fig. 1b), and expressed in *Atsr1-1* mutants. Most of the T1 plants (>30) complemented with 35S::*Atsr1 (I909V)* (cM1) showed a rescued phenotype. None of the >30 individual T1 plants complemented with either 35S::*Atsr1 (K907E)* (cM2) or 35S::*Atsr1 (A900–922)* (cM3) was restored to the wild-type growth level. For accurate comparison of all the complemented lines, T2 plants with verified genotype and similar transgene expression (Fig. 4b) were compared for their phenotypes. The *Atsr1-1* mutants complemented with 35S::*Atsr1* (cW) or cM1 plants were restored to wild type with regards to their morphology (Fig. 4c), growth (Fig. 4d) and disease resistance (Fig. 4e). The level of salicylic acid in *cW* and cM1 plants was ~60% of wild type (Fig. 4f), and expression of *PR* and salicylic acid signalling genes in *cW* and cM1 plants was also slightly lower than in wild type (Fig. 4g). However, cM2 and cM3 plants resembled the *Atsr1-1* plants with chlorosis (Fig. 4c) and slightly increased growth (Fig. 4d). The level of salicylic acid in cM2 and cM3 plants was slightly lower than that in *Atsr1-1* plants, but still markedly higher than that in wild-type plants (Fig. 4f). The level of disease resistance of cM2 and cM3 plants was slightly lower than that in *Atsr1-1* plants but significantly higher than in wild-type plants (Fig. 4g). The fact that *Atsr1* mutants that lost their CaM-binding activity are compromised in their function suggests that Ca²⁺/CaM-binding is required for Atsr1 to suppress plant immunity.

Plant immunity is a well-balanced process in which Ca²⁺-mediated signalling is actively involved. It is documented that Ca²⁺-mediated signalling stimulates the production of ROS and NO, which positively regulate hypersensitive response and plant defence. Positive regulators in the plant defence machinery are amplified in multiple
regulators such as AtSR1 is a challenging question. Recent studies revealed that interference with or removal of negative regulators are effective approaches in activating plant defence.\textsuperscript{12,26,30} Conceivably, interfering with the Ca\textsuperscript{2+} signal activating AtSR1, or AtSR1 itself, could also produce a quick activation of plant defence, a possibility that deserves further investigation.

**METHODS SUMMARY**

Arabidopsis knockout lines were obtained from Arabidopsis Biological Resource Center at Ohio State University. Knockout lines and primers for identification of homozygous knockout mutants are listed in Supplementary Table 1, and homozygous knockouts in single and double mutants were identified using polymerase chain reaction (PCR) analysis. *Pseudomonas syringae* was infiltrated into leaves of tested Arabidopsis plants for disease resistance assay or analysis of induced expression of PR genes. Hydrogen peroxide was detected with 3,3'-diaminobenzidine (DAB) staining, and autofluorescence was observed under a fluorescence microscope. Salicylic acid quantification was performed using high-performance liquid chromatography. Site-directed mutagenesis and deletion mutants of *AtSR1* were generated with PCR-based mutagenesis. Electrophoretic mobility-shift assay (EMSA) of DNA fragment, protoplast transient expression and chromatin immunoprecipitation were performed as described in Methods.

Wild-type and mutated AtSR1 proteins were expressed in *Escherichia coli* for CaM overlay assay or expressed in wild-type and *Atsr1* background for functional analysis using the floral dip approach. Gene expression was detected with northern blot, RT–PCR or western blot analyses depending on specific purposes.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Identification of Arabidopsis knockout mutants and generation of double mutants. Arabidopsis homozygous knockout plants were isolated from plants germinated from the BDCA DNA insertion collection using PCR analysis. The effects of the T-DNA insert on the interrupted genes were confirmed by northern blot analysis or RT-PCR. The primers used for analysis of mutants are listed in Supplementary Table 1.

Arabidopsis AtSR1 homozygous knockout line Atsr1-1 (Salk_001152) was crossed with css1/sid2 (Salk_088254), pad4 (Salk_089936) and eds2 (Salk_091541). Double mutants were selected from the F2 population by PCR analysis.

Pseudomonas syringae infection, time course induction and disease resistance assay. Pseudomonas syringae pv. tomato DC3000 culture and inoculation was performed as previously described [28, 29]. Briefly, leaves of 4–5-week-old plants grown at 25–27°C with 12-h photoperiod were infiltrated with Pst DC3000 at OD600 = 0.001 in 10 mM MgCl2 for time course induction and disease test. Leaves of 5-week-old plants grown at 19–21°C with 12-h photoperiod were infiltrated with Pst DC3000 at OD600 = 0.001 in 10 mM MgCl2 for disease resistance assay. Each disease resistant result is the average of four replicates; the results are presented as mean ± s.d.

Detection of H2O2 and autofluorescence. In situ H2O2 detection was performed essentially as described earlier [30]. Leaves from wild-type and mutant plants were vacuum-infiltrated with 1 mg ml–1 DAB (Sigma). The infiltrated leaves were incubated in the DAB solution for 6 h under high-humidity conditions. Leaves were fixed and cleared of chlorophyll with several changes of a 1:3:1 mixture of ethanol:acetone:water and dried as described above. Each disease resistant result is the average of four replicates; the results are presented as mean ± s.d.

Measurement of salicylic acid through HPLC. Salicylic acid quantification was performed as previously described with minor modification. Briefly, leaf tissue was collected from 5-week-old plants. For each sample, 150–200 mg tissue was ground in liquid nitrogen, and extracted with 90% methanol. After the extraction was dried, 500 μl 5% trichloroacetic acid was added to the residue. The free salicylic acid was extracted from the aqueous phase with ethylacetate-cyclopentane (1:1), and the organic phase was dried under nitrogen. The conjugated salicylic acid in the aqueous phase was hydrolysed at 100 °C in HCI solution with pH 1 for 30 min. The released free salicylic acid was extracted with organic mixture and dried as described above.

The dried extract was dissolved in 100 μl HPLC mobile phase, and 10 μl was injected into the HPLC column (SHERISORD ODS-2, 4.6 × 150 mm, 5 μm, Waters), and chromatographic separation was performed at 40 °C with a flow rate of 1.0 ml min–1. Salicylic acid was detected by a fluorescence detector.

Wild-type and mutated versions of AtSR1 cDNA and −1.5-kb EDS1 promoter. Full-length AtSR1 cDNA was isolated from an Arabidopsis ZAP Express (Strategene) by library screening. The full-length AtSR1 cDNA was cut from the pBK-CMV vector with BamHI and XbaI and cloned into a modified pBluescript II KS+ vector. The coding region was amplified with PCR, and EcoRI and XhoI sites were added at the ends of the fragments, and cloned into pET32A for stable transformation.

Recombinant protein purification, EMSA and 32P-CaM-binding assay. The E. coli strain BL21(DE3)/pLySs carrying the above pet32a-derived plasmids for expression of recombinant proteins of the wild-type and mutated versions of AtSR1, AtSR6 or ABF1 were induced with 0.5 mM IPTG for 3 h. 6×His-tagged recombinant proteins were purified using Ni-NTA agarose affinity beads (Qiagen) as described by the manufacturer. Recombinant AtSR1 or AtSR6 covering CG-1 domain, or ABF1, was used for EMSA [31] to detect its interaction with wild-type or mutated EDS1 promoter fragments. Recombinant AtSR1 containing CaMBD was used for CaM overlay assay [32].

Chromatin immunoprecipitation analysis. Thirty million leaf mesophyll protoplasts from 4-week-old wild-type Arabidopsis plants were transfected with 50 μg of YFP control DNA or AtSR1-YFP DNA with the PEG-mediated transformation method [33]. Protoplasts were incubated at 25 °C under dark conditions for 16 h before chromatin immunoprecipitation assay [34]. The harvested cells were resuspended in W5 medium containing 1% formaldehyde and crosslinked for 20 min. The protoplasts were lysed and DNA was sheared on ice with sonication. The pre-cleared lysate was incubated with 60 μl anti-GFP agarose beads (D153-8, MBL) for 12 h at 4 °C. Beads were washed five times, re-suspended in elution buffer and incubated at 65 °C for 12 h. After purification, the DNA was amplified with PCR using EDSEU-F (5′-TGCCTTTGCTAGAATTTTCCC-3′) and EDSEU-R (5′-GGACCGTTTGATTCTTGCTC-3′) primers.

eds1::luc transient expression assays. One-million protoplasts from 4- to 5-week-old wild-type and Atsr1-1 plants grown at 20 °C were transfected in four replicates with 5 μg GUS plasmid (as internal control) and 5 μg pDL326 or pDL327 plasmids with the PEG-mediated transfection method [33]. After 16–17 h incubation at 20 °C, the protoplasts were harvested and luciferase assays were performed using a lucerase assay kit (Promega). To account for variation in transfection efficiencies, Gus assays were performed with each treatment using standard methyl umelliferyl glucuronide substrate. The data presented are the average of the Luc/GUS ratios of four replications ± s.d.


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Ca\textsuperscript{2+}/calmodulin Regulates Salicylic Acid-mediated Plant Immunity


*To whom correspondence should be addressed. Email: Poovaiah@wsu.edu

**Supplementary table 1:** list of primers for characterizing Arabidopsis mutants

<table>
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<th>Gene</th>
<th>mutant line</th>
<th>L primer</th>
<th>R primer</th>
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<tr>
<td>AtSR1</td>
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<td>GAACTACTGAACATTTTCTAGAAGTTACTCAC</td>
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<td>Salk_088254</td>
<td>ACTTATTTTTCTGGCCCAAAAAAC</td>
<td>CACTTTACGAATTTCCTGCAATGG</td>
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Supplemental Figure 1. Schematic illustration of *AtSR1/CaMTA3* and its complementation constructs. A. Endogenous *AtSR1*, T-DNA insert in Salk_001152 (atsr1-1) and Salk_064489 (atsr1-2) lines, primers used for checking T-DNA insert, knockout status, and RT-PCR are indicated, AtSR1-L: GAACTACTGAACATTTTCTAGAAGTTACTCAC, AtSR1-R: TGTTTGGGCAAACAGAAGTTC, LBa1 sequence was previously described, P1: CCATCCATGTCCCTCCTAGA, P2: TCCATTGATTCCCAAACCTG, P3: TTCAGCCCGTTCATGAATTAG. B. Complementation constructs of *AtSR1/CaMTA3* and its mutants. The CaMBD is enlarged, nucleotide and amino acid sequences of wild-type CaMBD are in blue, the mutated positions are in red and underlined, deleted parts are joined with a bent line. The first mutation I909V (M1) does not disrupt the conserved secondary structure of the AtSR1 CaMBD. The second mutation K907E (M2) drastically alters the surface static charge of its CaM-binding helix. The third mutation (M3) A900-922 is a deletion of the whole CaMBD from aa 900 to 922 of AtSR1.
Supplemental Figure 2

A. Molecular characterization demonstrating the genotype of WT and atsr1-1. PCR1: DNA amplified with AtSR1 specific primer AtSR1-R and T-DNA specific primer Lba1; PCR2: DNA amplified with AtSR1 specific primer AtSR1-L and AtSR1-R (see Fig. S1a for primer sequences). AtSR1 probed: Northern blot shows the expression of AtSR1 gene in both WT and atsr1-1 knockout mutant, EtBR stained rRNA was used as loading control (rRNA).

B. Phenotypic comparison. 7-week-old WT and atsr1-1 mutant plants grown at 19-21°C with 12 hr photoperiod.

C and D. Phenotypic comparison. Five-week-old WT (C) and atsr1-2 mutant (D) plants grown at 19-21°C with a 12-hr photoperiod.

E. Genotype of atsr1-2 was confirmed by RT PCR with AtSR1-specific primers (see Table 1). AtSR1 transcript was shown to be absent in the atsr1-2 mutant by RT-PCR using two pairs of primers: one on either side of the insertion and one downstream of the insertion (Fig. S1a). Expression of cyclophilin (At4g38740) was used as loading control.
Supplemental Figure 3: A. Staining of similar age leaves from uninfected WT, *atsr1-1* and WT inoculated with a 10^5 CFU mL^{-1} suspension of *Pseudomonas syringae* pv *tomato* carrying AvrRpt2 with 3,3’-diaaminobenzidine (DAB) reveals accumulation of H_2O_2 in *atsr1* and WT inoculated with *Pst* AvrRpt2. B. **Quantification of DAB stains** per 2.5 mm^2. Each bar is the average of at least four leaves. Error bars represent SD.
Supplemental Figure 4. **Functional complementation and overexpression of AtSR1.** All the plants used in these experiments were grown at 19-21°C with 12 hr photoperiod. **A. 5-week-old plants** of wild-type (WT), atsr1-1 mutant (atsr1-1), atsr1-1 complemented with wild-type AtSR1 gene (cW), and two overexpression (OE) lines of AtSR1 gene with different transgene expression levels (OE1 and OE2). **B. Molecular characterization** demonstrating the genotype of the plant lines listed in “A”. PCR1, PCR2: see “legend of Fig. S2A”. atsr1-1 is marked as atsr1 in all panels hereafter. α-FLAG: 20 µg of total protein from each sample was used in the Western blot detected with anti-Flag M2 monoclonal antibody (Sigma). **C. PRI expression** in plant lines as listed in “A”. **D. Growth comparison** in terms of fresh weight measured at five weeks, compared plant lines are the same as listed in “A”. **E. Disease Resistance Test.** Pst. DC3000 (0.0001 OD600) was infiltrated into the rosette leaves, and the cfu was measured 3 days after infiltration. Data are expressed as mean ± s.d (n=4, *p<0.045 by T-test).
Supplemental Figure 5. Pathogen induced SA accumulation in WT and atsr1-1(atsr1) grown at 25-27°C. Leaves of 5-week-old plants grown at 25-27°C with 12 hr photoperiod were infiltrated with Pst. DC3000 at OD$_{600}$=0.001. Infected leaves were collected at the indicated times after inoculation and used for free SA quantitation. Each result is expressed as mean ± s.d. (n=4).
Supplemental Figure 6

A. Expression of key SA signaling and synthetic genes in wild-type (WT) and atsr1-1 (atsr1, hereafter). Identical blots were hybridized to PAD4, EDS1, EDS5 and ICS1 probes, EB stained rRNA was used as loading control (rRNA).

B. Northern blots showing loss-of-function mutation in pad4 and ics1 knockout backgrounds. RNA samples were prepared from 5-week-old plants. Genotypes are marked beneath and probes to the right of the panel. EB stained rRNA was used as loading control (rRNA).

C. Impact of SA signaling and synthesis mutants on disease resistance of atsr1. Pst. DC3000 (OD\textsubscript{600} 0.0001) was infiltrated into the leaves of 5-week-old plants grown at 19-21°C, and the cfu was measured 3 days after infiltration. The results of 4 replicates were averaged. Genotypes are marked beneath the panel.

D. PR1 expression in compared plants. RNA samples were prepared from 5-week-old plants grown at 19-21°C, and the RNA blot was hybridized to PR1 probe. EB stained rRNA was used as loading control (rRNA). Genotypes are marked beneath the panel.

E. Comparison of plant growth. 5-week-old plants grown at 19-21°C. Genotypes are marked beneath the panel.
Supplemental Figure 7

A. Endogenous EDS1 with promoter

B. Example of Possible TFs Binding ACGCGT box. There are other TFs in the Arabidopsis genome which might bind to ACGCGT motif in EDS1 promoter. Possible candidates include other AtSR homologs, ABFs (ABRE binding factor) which recognize ACGCGT-like motif. AtSR6 and ABF1 was selected for testing.

EDS1 promoter fragment (EDS1P, GTAAAAGTCGAATGTGACGCGTCTTGCCGAAC) or a mutated version with its ACGCGT changed to ACCCGT (eds1p) was labeled as probe, recombinant AtSR6 contains a CG-1 DNA-binding domain, ABF1 (ABRE binding factor 1) is a full-length recombinant protein.

C. Regulatory model of EDS1 promoter. Negative regulator (-), AtSR1, competes for the CGCG box with unidentified TF(s), a positive regulator (+), required for the normal function of EDS1 promoter. In WT, because of the balanced action of AtSR1 and the unknown TF, EDS1 is slightly expressed (upper panel). In the absence of AtSR1, the repression of AtSR1 is removed, and EDS1 expression is activated (middle panel). When the CGCG box was mutated, the promoter lost its response to positive, as well as negative regulation because the critical unknown TF could no longer bind to EDS1 promoter (lower panel).
Supplemental Figure 8

A. 6-week-old 

B. 6-week-old 

C. Northern analysis of independent rescued M326 lines probed with PAD4, EDS1 EDS5, ICS1 or PR1. EtBR stained rRNA was used as a control.

References