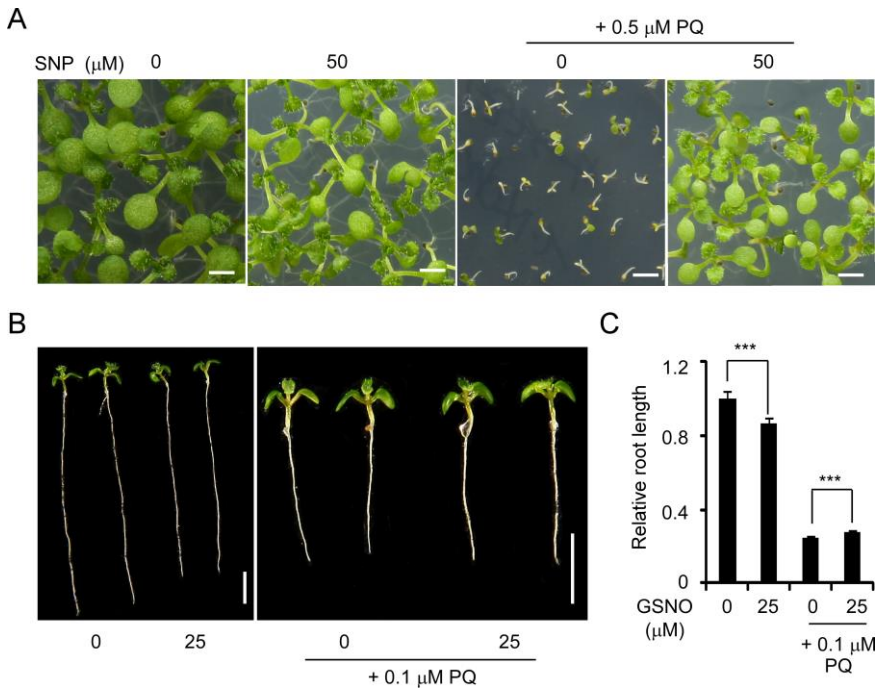
SUPPLEMENTAL DATA

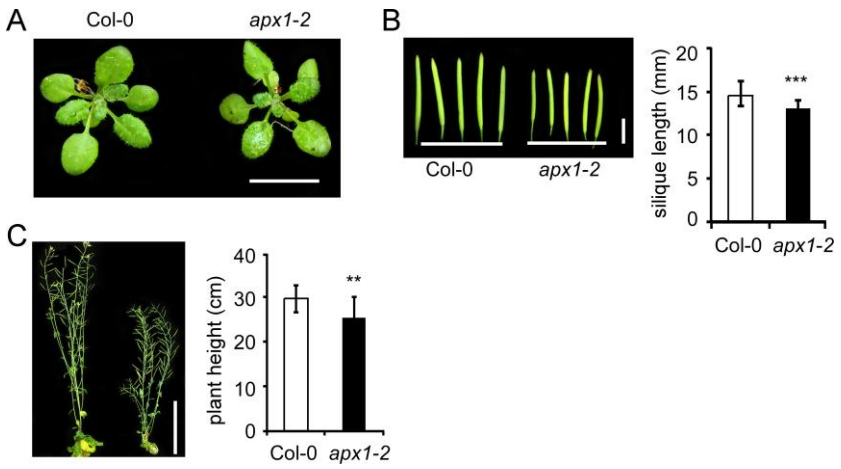


Supplemental Figure S1 Nitric oxide enhances the resistance to oxidative stress.

A, Seven-day-old Col-0 seedlings treated with various combinations of paraquat (PQ; 0.5 μM) and sodium nitroprusside (SNP). Scale bars, 0.5 cm.

B, Ten-day-old Col-0 seedlings treated with various combinations of GSNO and paraquat (PQ) (0.1 μM). Scale bars, 0.5 cm.

C, Analysis of the relative length of the primary roots shown in (B). The relative root length of Col-0 seedlings grown under normal growth condition is set at 1.0. At least 30 seedlings were used for each sample. Error bars indicate SD from three independent experiments (biological replicates). Two-tailed student's *t*-test, *** indicates $P < 0.001$.



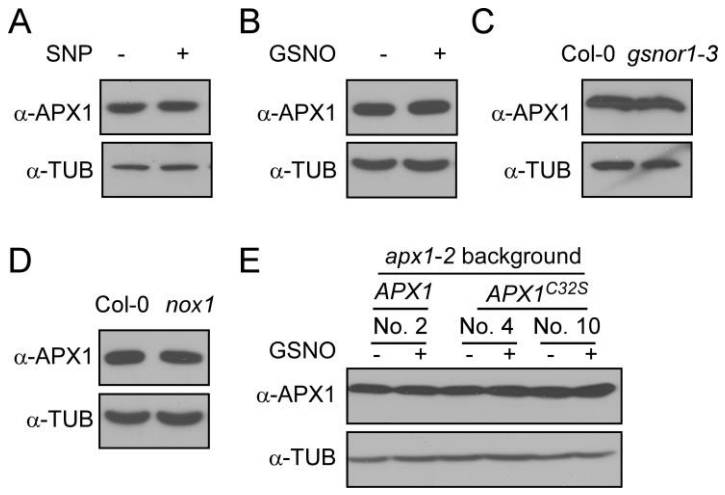
Supplemental Figure S2 The *apx1-2* mutant phenotype.

A, Four-week-old Col-0 and *apx1-2* seedlings grown under 16h light/8h dark cycle. Scale Bar, 1 cm.

B, Siliques derived from 13-week-old Col-0 and *apx1-2* plants. Bar, 5 mm. Quantitative analysis of the siliques length shown at the right (n = 30).

C, Thirteen-week-old Col-0 and *apx1-2* mutant plants. Bar, 5 cm. Quantitative analysis of the plant height shown at the right (n = 30).

Error bars in (B) and (C) indicate SD from three independent experiments (biological replicates). Two-tailed student's *t*-test, ** and *** indicate $P < 0.01$ and $P < 0.001$, respectively.



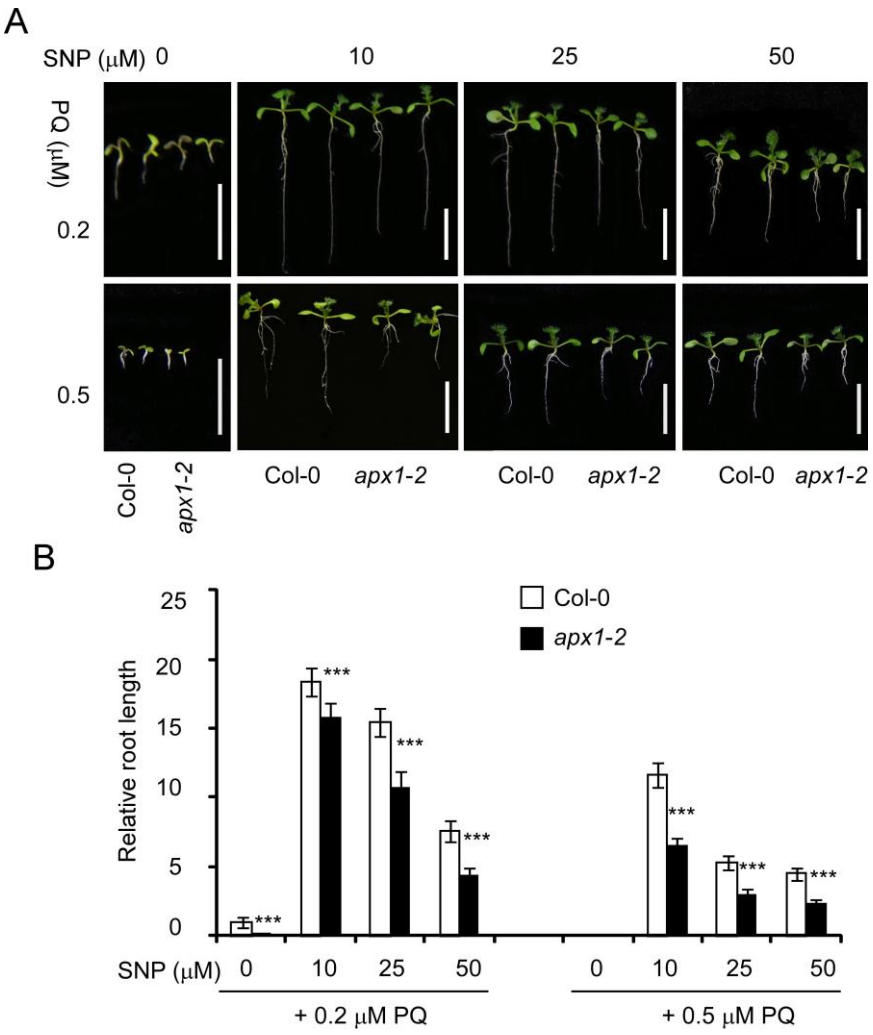
Supplemental Figure S3 Analysis of the accumulation of APX1 protein.

A and B, Accumulation of APX1 protein in Col-0 treated with 50 μM sodium nitroprusside (SNP) (A) or 200 μM GSNO (B).

C and D, Accumulation of APX1 protein in Col-0, *gsnor1-3* (C) and *nox1* (D) seedlings.

E, Accumulation of APX1 protein in transgenic plants with the indicated genotypes (numbers refer to the transgenic lines).

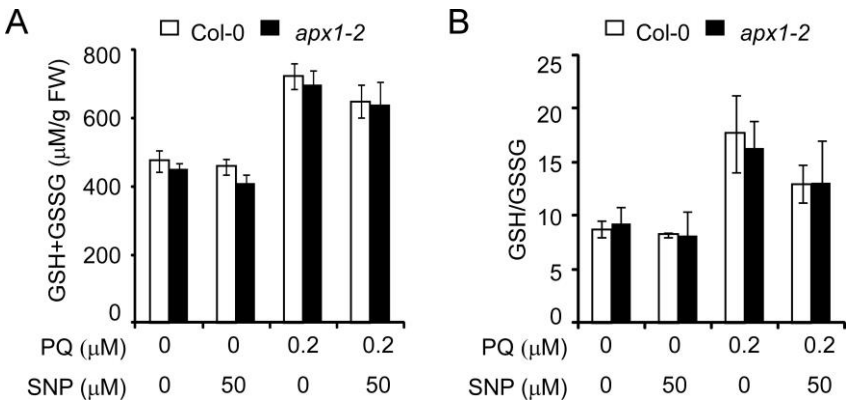
APX1 protein was detected by immunoblotting using an anti-APX1 antibody. Equal loading was verified using an anti-TUBULIN antibody.



Supplemental Figure S4 Sodium nitroprusside enhances the resistance to oxidative stress.

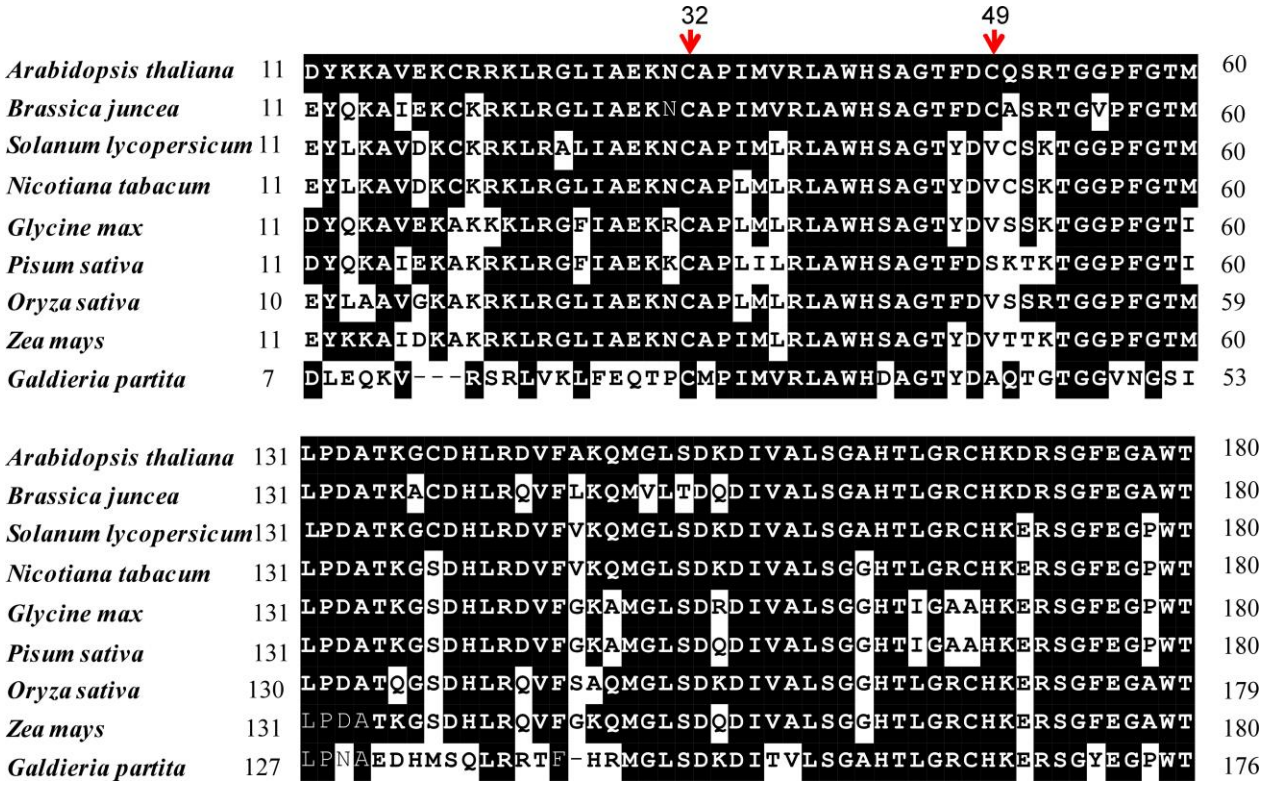
A, Ten-day-old seedlings of Col-0 and *apx1-2* germinated and grown on 1/2 MS agar plates supplemented with various combinations of sodium nitroprusside (SNP) and paraquat (PQ). Scale bar, 1 cm.

B, A quantitative analysis of the relative root length shown in (A) (n = 30). The relative root length of Col-0 seedlings grown under normal growth condition is set at 1.0. Error bars indicate SD from three independent experiments (biological replicates). Two-tailed student's t-test, *** indicates $P < 0.001$.



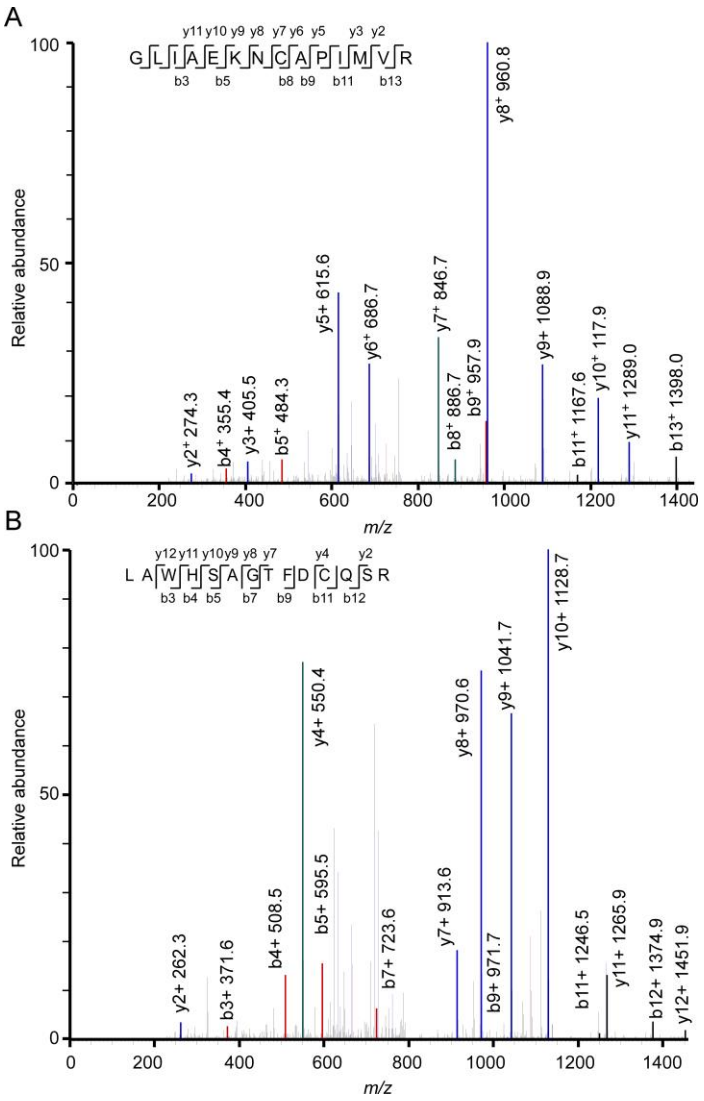
Supplemental Figure S5 Analysis the GSH and GSSG level.

A and B, Analysis the GSH+GSSG content (A) and the GSH/GSSG level (B) in 10-day-old seedlings of Col-0 and *apx1-2* germinated and grown on 1/2 MS agar plates supplemented with various combinations of sodium nitroprusside (SNP) and paraquat (PQ). Error bars indicate SD from three independent experiments (biological replicates).



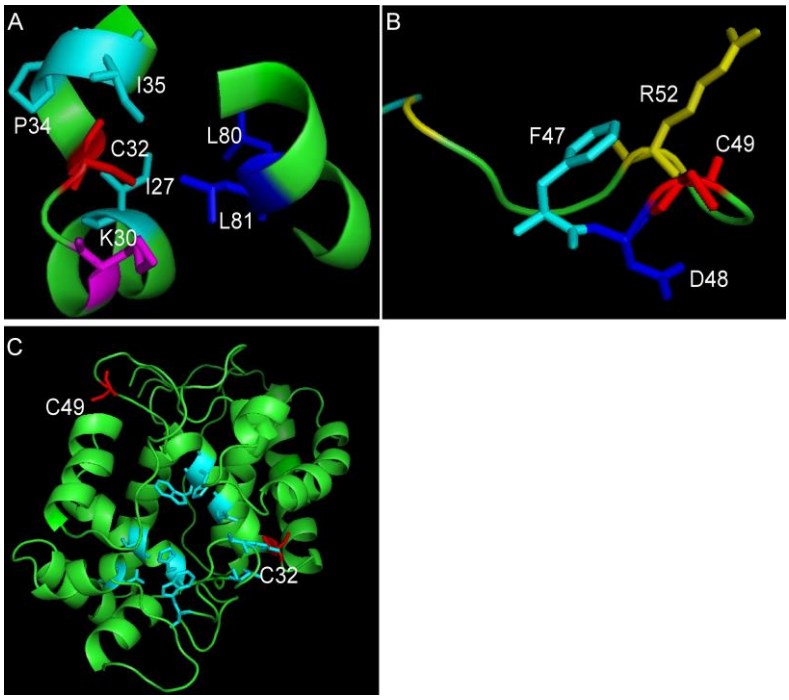
Supplemental Figure S6 Sequence alignment of APX proteins from various species.

Comparison of the partial sequences of Arabidopsis APX1 with APX proteins of other plant species and red alga *Galdieria partita*. The *S*-nitrosylated cysteine residues in Arabidopsis APX1 are highlighted by red arrows. These cysteine residues and their flanking sequences perfectly or partially matched the *S*-nitrosylation consensus characterized as an acid-base/hydrophobic motif. Accession numbers of the proteins: *Arabidopsis thaliana*: NP_172267.1; *Brassica juncea*: AAB94927.1; *Solanum lycopersicum*: NP_001234782.1; *Nicotiana tabacum*: BAA12918.1; *Glycine max*: NP_001237785.1; *Pisum sativum*: P48534.2; *Zea mays*: NP_001152746.1; *Oryza sativa*: XP_006658179.1; *Galdieria partita*: BAC41199.



Supplemental Figure S7 Mass spectra of APX1 peptides.

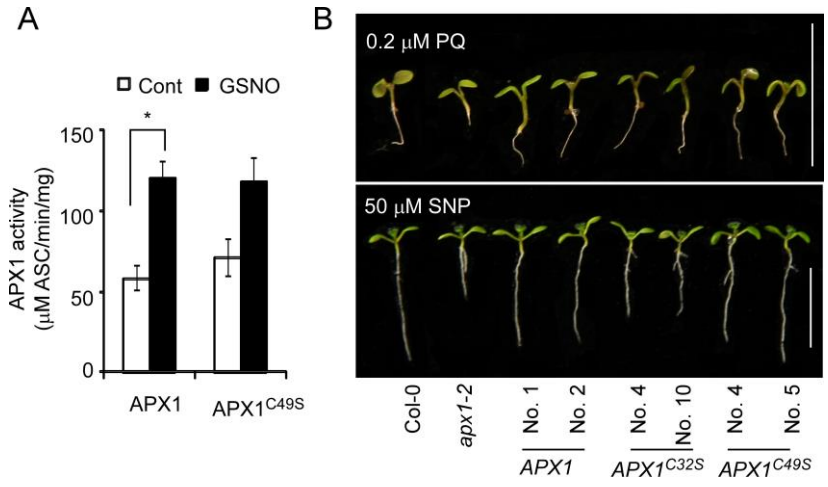
A and B, tryptic peptides containing Cys^{32} (A) and Cys^{49} (B) derived from native His-APX1 recombinant protein identified by mass spectrometry.



Supplemental Figure S8 Structural modeling of Arabidopsis APX1.

A and B, The putative local structures of APX1 around Cys^{32} (A) and Cys^{49} (B). The surrounding residues of Cys^{32} and Cys^{49} are also shown, which represent typical acid-base/hydrophobic motifs for *S*-nitrosylation.

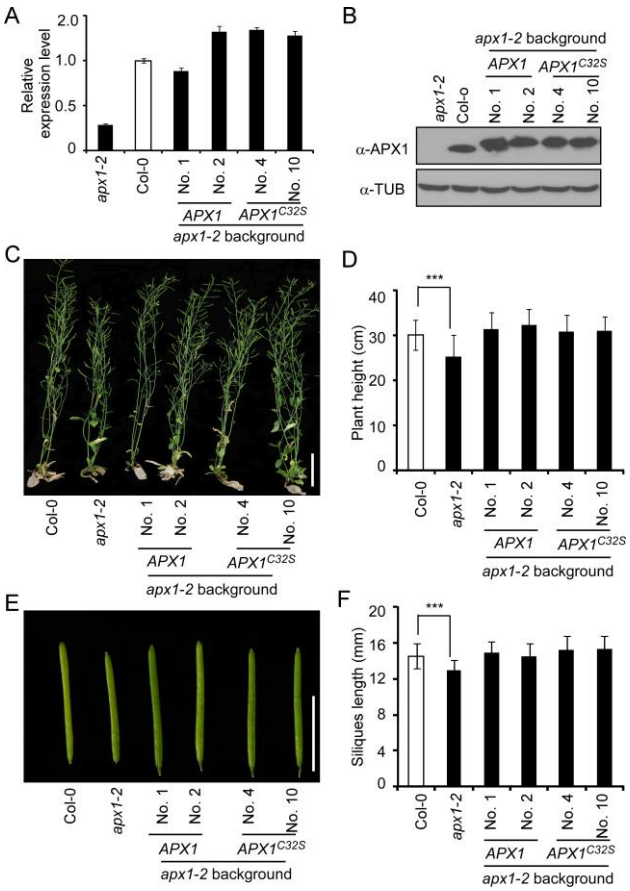
C, The putative structure of APX1. Cys^{32} is adjacent to the enzymatic active center (indicated as blue). The modeling was performed using the *Glycine max* APX1 protein structure (PDB code: 1OAF) as a training template.



Supplemental Figure S9 Functional characterization of Cys^{49} of APX1.

A, Analysis of the enzymatic activity of APX1 and APX^{C49S} recombinant proteins. Error bars indicate SD from three independent experiments (biological replicates); * indicates $P < 0.05$ (Student's *t*-test).

B, Analysis of the phenotype of transgenic plants carrying various transgenes with the indicated genotypes (all transgenics were in the *apx1-2* background).



Supplemental Figure S10 Characterization of *APX1* transgenic plants.

A, Analysis of *APX1* expression in 10-day-old seedlings with the indicated genotypes (numbers refer to the transgenic lines) by RT-qPCR.

B, Analysis of APX1-FLAG proteins in10-day-old seedlings with the indicated genotypes by immunoblotting using an anti-APX1 antibody.

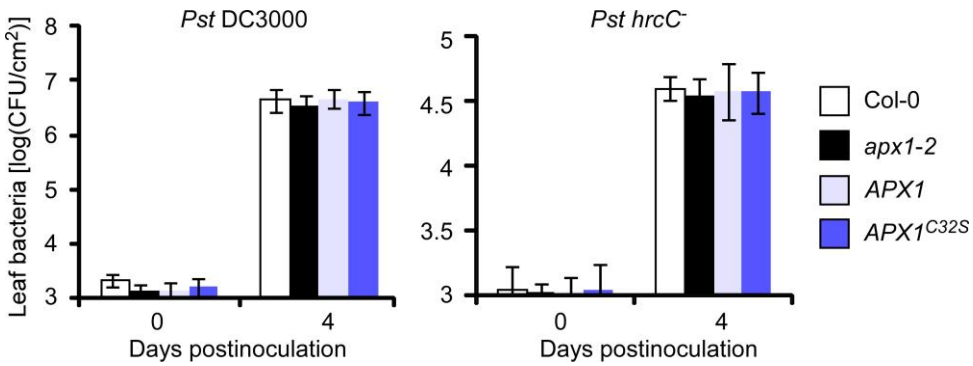
C, Nine-week-old plants grown under continuous white light. Bar, 5 cm.

D, Quantitative analysis of the plant height shown in (C). $n = 30$.

E, Siliques derived from plants shown in (C). Bar, 1 cm.

F, Quantitative analysis of the siliques length shown in (E). $n = 30$.

Error bars in (D) and (E) indicate SD from three independent experiments (biological replicates). Two-tailed student's *t*-test, ** and *** indicate $P < 0.01$ and $P < 0.001$, respectively.



Supplemental Figure S11 Analysis of bacterial growth in *apx1-2* and transgenic plants. Leaves derived from 4-week-old plants with the indicated genotypes were inoculated with *P. syrigae* DC3000 and *hrcC*- bacteria cell cultures. The bacterial population in the leaf was determined 4 days post-inoculation. Error bars indicate SD from eight technical replicates.

Supplemental Table S1 Primers used in this study

Primer	Sequences (5’ to 3’)	Experiments
APX1F1	GGGATCCATGACGAAGAACTACCCAACCGTG	His-APX1
APX1B1	GGTCGACTTAAGCATCAGCAAACCCAAGCTC	His-APX1
APX1F2	CCTCGAGACACGACTTCCGTCGAGTAGATTC	APX1 genomic DNA
APX1B2	GCCCGGGAGCATCAGCAAACCTGCAACCAAC	APX1 genomic DNA
APX1F3	GTTGAAGTTACTGGTGGCCCTGA	Genotyping
APX1B3	AGCAGCGTATTTCTCGACCAAAGG	Genotyping
APX1F4	GTTGAAGTTACTGGTGGCCCTGA	Genotyping
APX1B4	CACACACACAGAGCATACGTCACAGC	Genotyping
APX1C32SF	TTTGATCGCTGAGAAGAACTCTGCACCCATCA	$APX1^{C32S}$
APX1C32SB	GAGTTCTTCTCAGCGATCAAACCTCTGAGCT	$APX1^{C32S}$
APX1C49SF	CTCTGCTGGAACTTTCGATTCTCAATCAAGG	$APX1^{C49}$
APX1C49SB	GAATCGAAAGTTCCAGCAGAGTGCCAT	$APX1^{C49S}$
UBQ5F	CTTCAGCAGCCGTTGCCTCA	RT-PCR
UBQ5B	CTGGTAAACGTAGGTGAGTCC	RT-PCR
ACT7F2	TCCATGAAACAACTTACAACTCCATCA	qRT-PCR
ACT7B2	CATCGTACTCACTCTTTGAAATCCACA	qRT-PCR
FRK1F	CGGTCAGATTTCAACAGTTGTC	qRT-PCR
FRK1R	AATAGCAGGTTGGCCTGTAATC	qRT-PCR
NHL10F	TTCCTGTCCGTAACCCAAAC	qRT-PCR
NHL10R	CCCTCGTAGTAGGCATGAGC	qRT-PCR