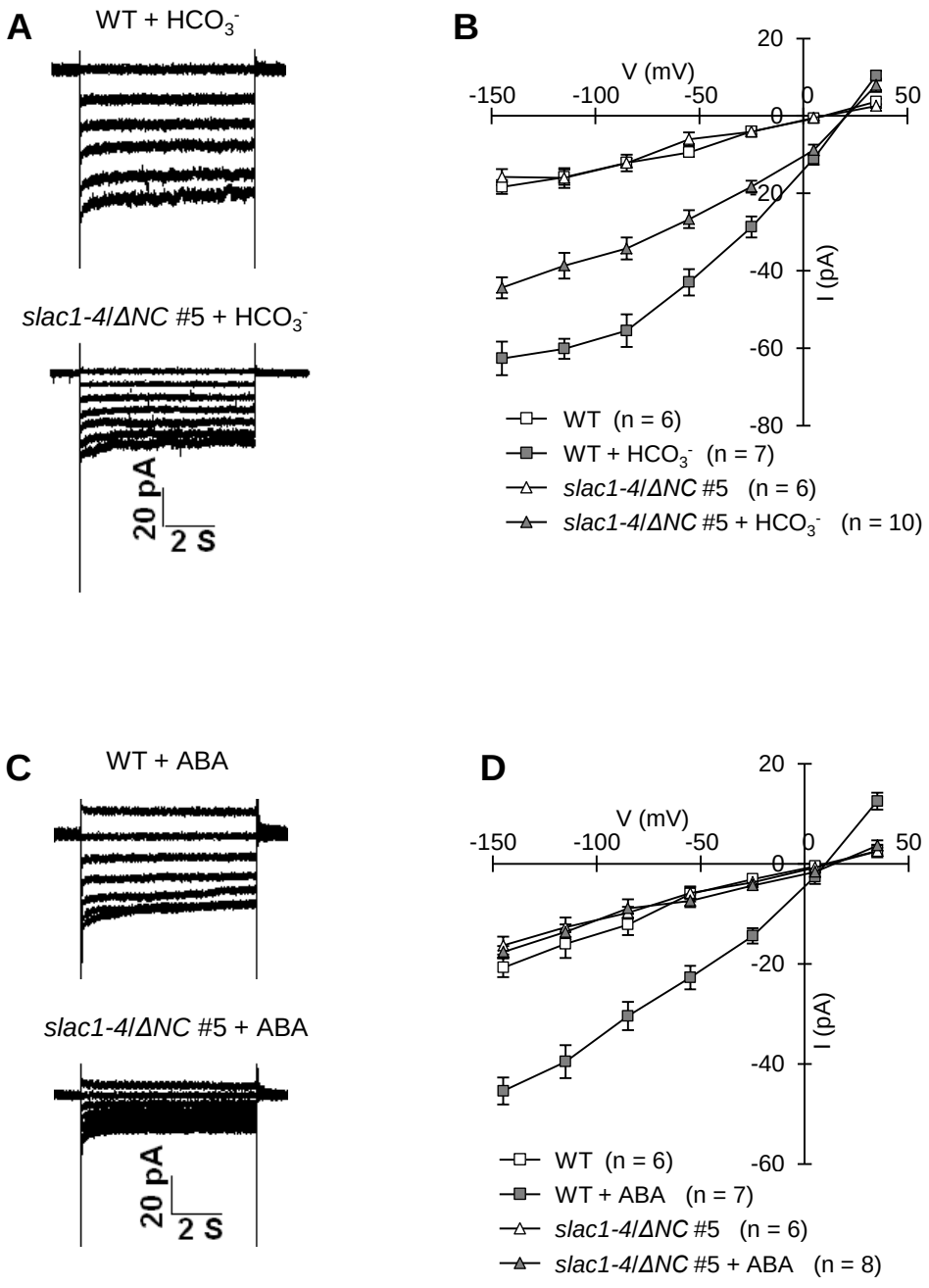
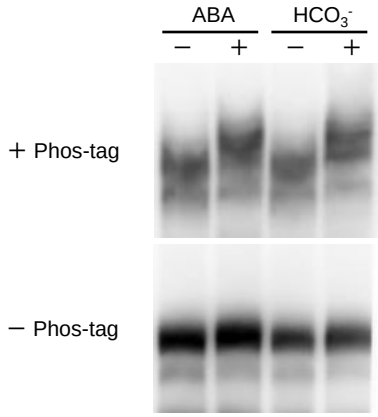
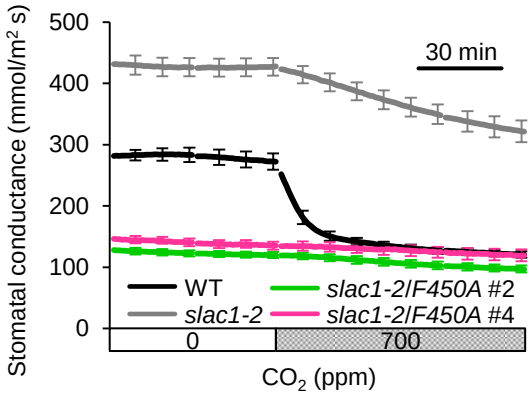


Supplemental Figure 1. Bicarbonate Activation of the S-Type Anion Currents Is Restored in Guard Cells of an Independent ΔNC Transgenic *slac1-4* Line, whereas ABA Activation of S-Type Anion Currents Is Impaired.
(A) Whole-cell currents in guard cell protoplasts of wild-type (WT) and the ΔNC transgenic line (*slac1-4*/*ΔNC* #5) with bicarbonate. **(B)** Steady-state current–voltage relationships of the whole-cell currents recorded in WT (squares) and the ΔNC transgenic line (triangles) with (closed symbols) or without (open symbols) bicarbonate. Error bars indicate ± SEM; n = 6–10 guard cells. **(C)** Whole-cell currents in guard cell protoplasts of WT and the ΔNC transgenic line line (*slac1-4*/*ΔNC* #5) with ABA. **(D)** Steady-state current–voltage relationships of the whole-cell currents recorded in WT (squares) and the ΔNC transgenic line (triangles) with (closed symbols) or without (open symbols) ABA. Error bars indicate ± SEM; n = 6–8 guard cells.

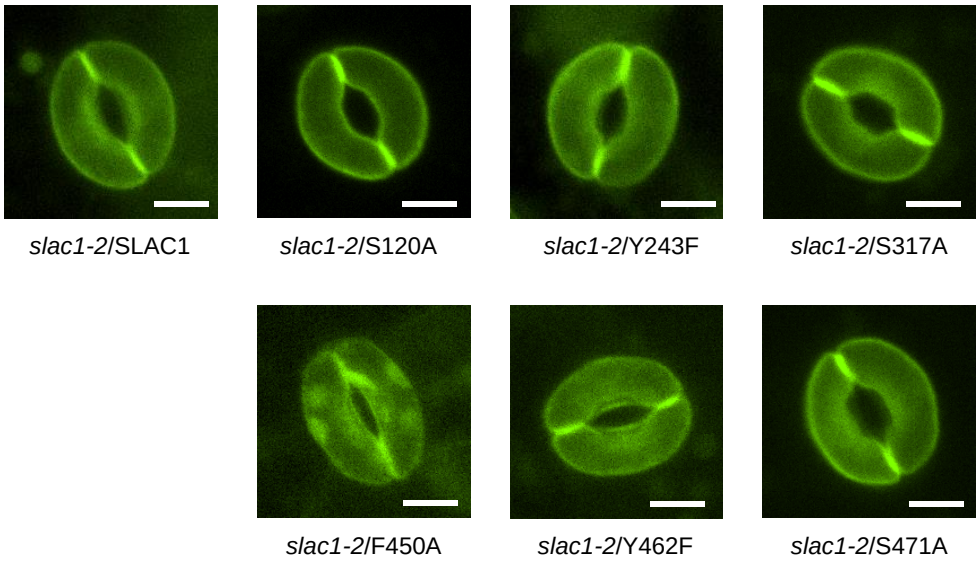


Supplemental Figure 2. The Mobility Shift of SLAC1 Protein Treated with Bicarbonate or ABA in SDS-PAGE Gels Containing Phos-tag.

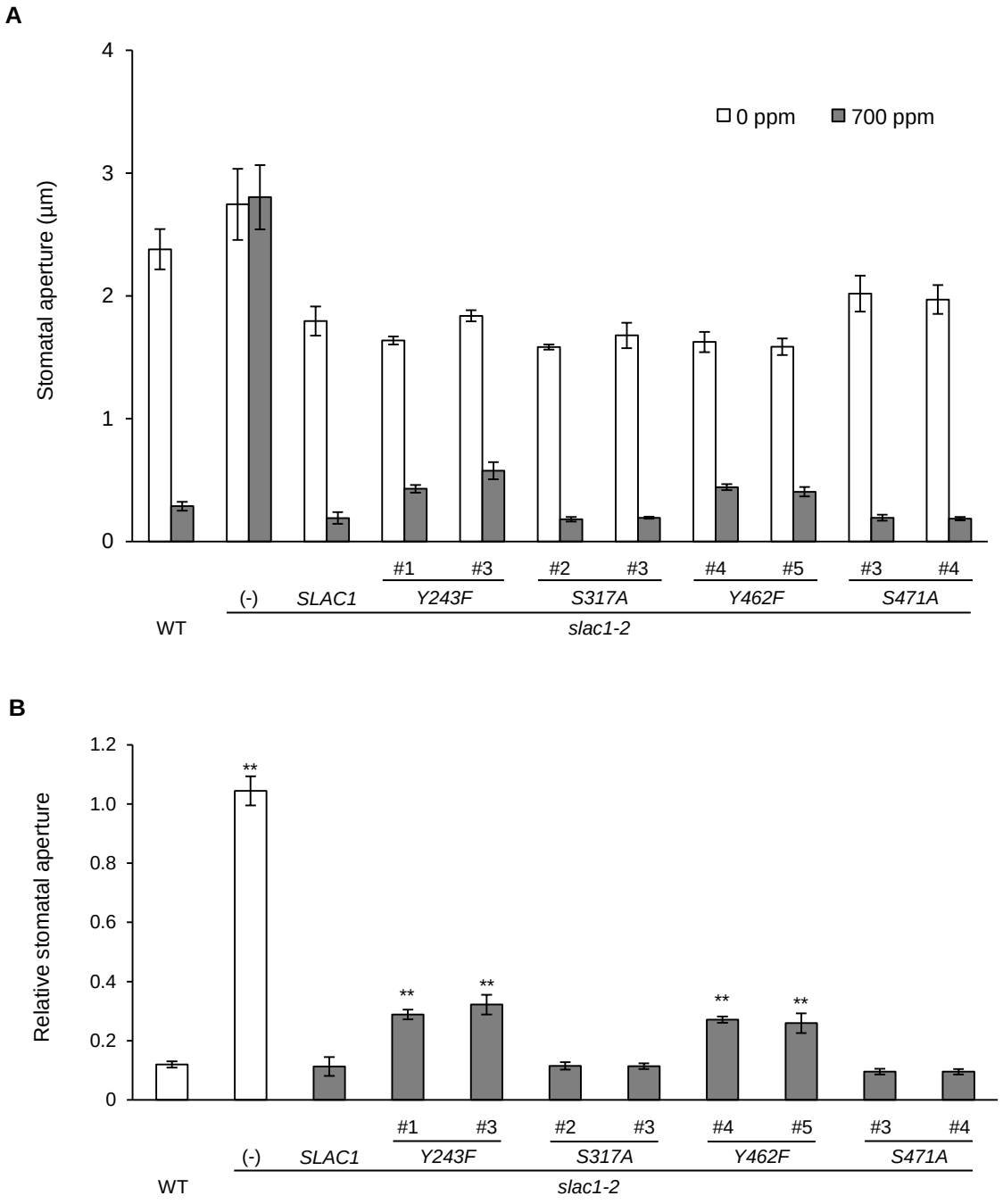
For this analysis, proteins were extracted from GCPs prepared from the transgenic plants expressing the SLAC1-GFP fused protein after treatment with 13.5 mM bicarbonate or 10 μM ABA. Protein samples were separated by SDS-PAGE containing Phos-tag (+ Phos-tag), a ligand that shifts the mobility of phosphorylated proteins, and analyzed by immunoblotting using a GFP antibody. The mobility of the SLAC1-GFP protein treated with bicarbonate was retarded in a manner similar to that prepared from GCPs treated with ABA. Protein samples were also separated in SDS-PAGE gels without Phos-tag (- Phos-tag).



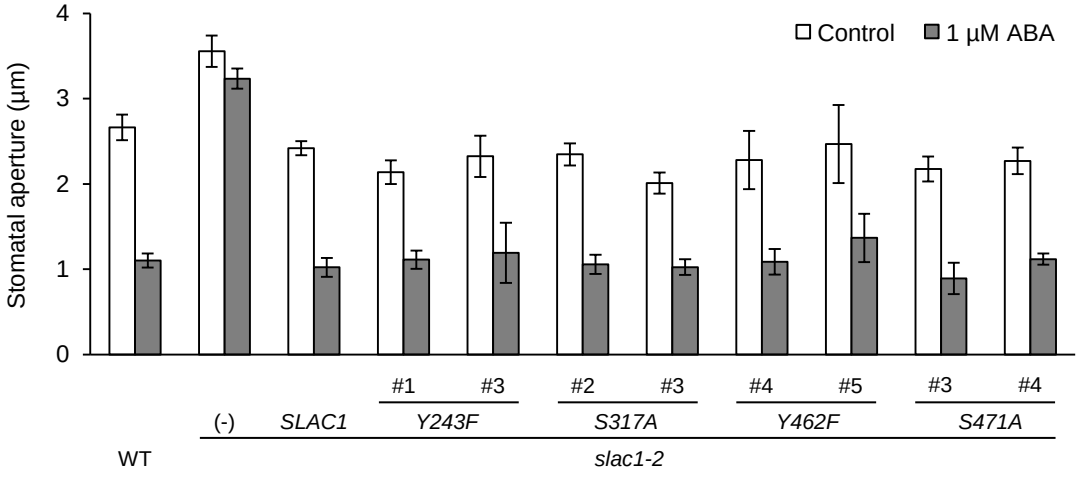
Supplemental Figure 3. Stomatal Response of Transgenic Plants Expressing SLAC1 F450A.
Time-resolved stomatal conductance in response to [CO_2] in wild-type (WT; black), the *slac1-2* mutant (light gray) and transgenic plants expressing SLAC1 F450A (magenta or green).
The transgenic plants had constitutively low levels of stomatal conductance. Plants were kept under constant white light of 150 µmol m^{-2} s^{-1} at 22 ^{o}C in 45% relative humidity. Error bars indicate ± SEM; n = 3–5.



Supplemental Figure 4. Subcellular Localization of Mutant SLAC1 Proteins in the *slac1* Transgenic Plants. Fluorescence microscope images of GFP fused to SLAC1 and its mutated variant proteins in guard cells. Because of low expression of F450A protein, exposure times for *slac1-2*/F450A line were five times longer than for the other lines. An auto-fluorescence signal of chloroplast in F450A line occurs as a result of the long time exposure. Bars = 5 µm.



Supplemental Figure 5. Stomatal Aperture of the Transgenic Lines in Response to CO_2.
(A) CO_2 regulation of stomatal aperture in the wild-type (WT), the *slac1-2* mutant and transgenic lines expressing mutant SLAC1 protein. White bars indicate low CO_2 (0 ppm) and gray bars indicate high CO_2 (700 ppm) conditions. Two independent transgenic lines per construct were analyzed. **(B)** Relative stomatal aperture of the transgenic lines. Apertures at 700 ppm were normalized with those at 0 ppm. Asterisks denote comparison with wild-type *SLAC1* complementation line: **P < 0.05 by one-way ANOVA with Dunnett's *post hoc* test. Plants were kept under constant white light of 42 µmol m^{-2} s^{-1} at 22 °C in 45% relative air humidity. Error bars indicate ± SEM; Data from four independent experiments were averaged.



Supplemental Figure 6. Stomatal Aperture of the Transgenic Lines in Response to ABA.
ABA regulation of stomatal aperture in the wild-type (WT), the *slac1-2* mutant and transgenic lines expressing mutant SLAC1 protein. White and gray bars indicate stomatal apertures in the absence (control) and presence of 1 µM ABA. Two independent transgenic lines per construct were analyzed. Error bars indicate ± SEM; data from four to five independent experiments were averaged.

Supplemental Table 1. Oligonucleotide Primers Used in This Study.

Oligo Name	DNA sequence (5'-to-3')
SLAC1 promoter cloning	
pSLAC1-F (Sal Ⅰ)	CTTGTCGACGATGCGCTCTACGA
pSLAC1-R (Nco Ⅰ)	GTTTCCTCTCCATGGATCAGAGC
ΔN3 cloning	
ΔN-F (Nco Ⅰ)	CTCCATGGAGCAATGGCCGTTTCTCCT
SLAC1:GFP-R (Nco Ⅰ)	GACCATGGCTCCACCTCCACCGTGATGCGACTCTTCCTC
pSLAC1:ΔC cloning	
pSLAC1-F (Sal Ⅰ)	CTTGTCGACGATGCGCTCTACGA
ΔC:GFP-R (Nco Ⅰ)	GACCATGGCTCCACCTCCACCATCGTTTGGGAACAACGTTT
ΔNC cloning	
ΔN-F (Nco Ⅰ)	CTCCATGGAGCAATGGCCGTTTCTCCT
ΔC:GFP-R (Nco Ⅰ)	GACCATGGCTCCACCTCCACCATCGTTTGGGAACAACGTTT

Supplemental Table 2. DNA Sequences of the Amino acid Substitutions.

Lines	DNA sequence (5'-to-3')
S120A	CAAACAAAAGgcaTTATTGCCTT
Y243F	GTCCTTCACTttcATACTCAAAT
H260F	AGAGTATTTTttcCCTGTTCGAG
S317A	GCAATGGCTTgcaGGAGGGAAGA
F450A	GTCATATACTgctCCTATGACAAC
Y462F	AACAATAAAGtttGCAGAGGCAG
S471A	TGGTTACCCGgctCGGGCTCTAG

The mutagenic codons are indicated by lowercase letters.