

Supporting Information

Title: Cytosolic acidification and oxidation are the toxic mechanisms of SO₂ in Arabidopsis guard cells

Short title: SO₂ toxicity in plant cells

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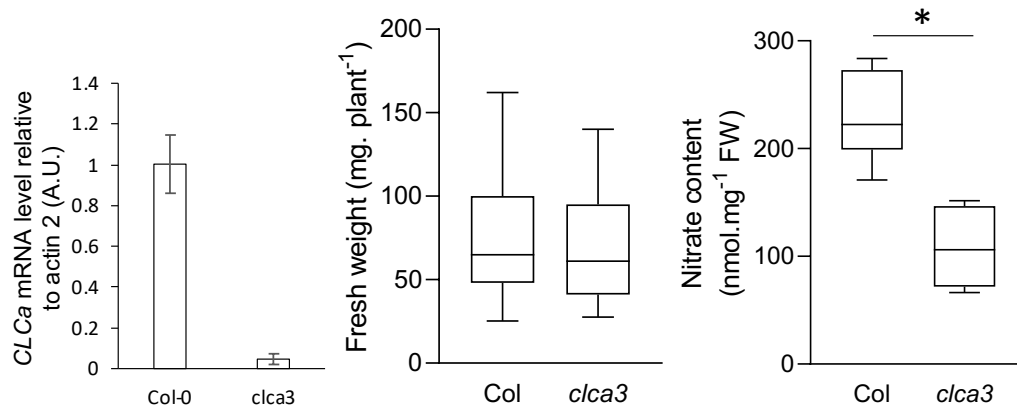
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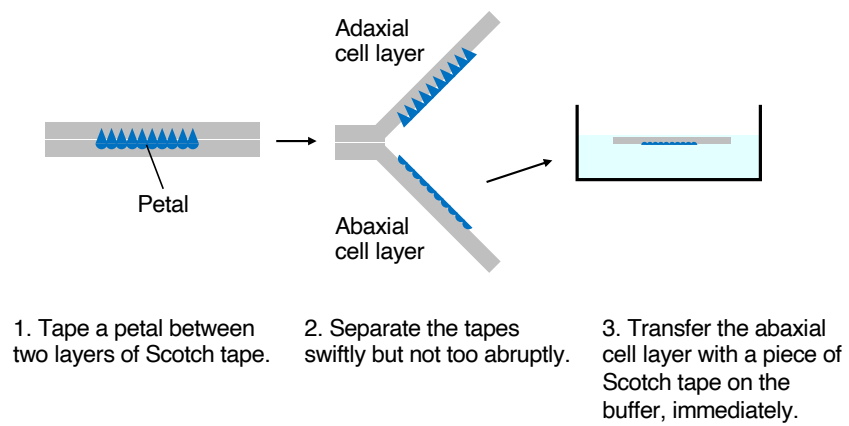
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Supplementary Table S1. Nucleotide sequences of primers used in this study

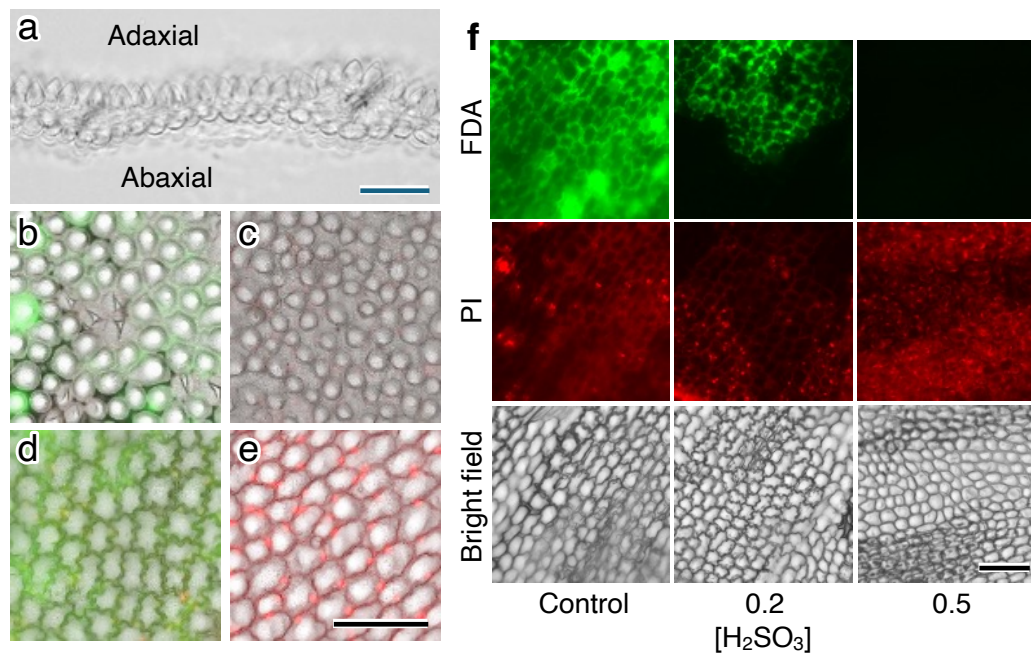
Primer name	Nucleotide sequence (5' to 3')
CLCa-F	CTGACTTTGGTGGCGACAGTACTTGTT
CLCa-R	ACGGCTGTACTGAAGAATGTTCTCCAC
CLCb-F	CATCGCCGGCTATAAGCTTTTAGCCGT
CLCb-R	CACTCCTGCAGCTGAGCCACATGTAAT
CLCc-F	CAACTTCTCAGATCGCTATTGTCGGAG
CLCc-R	CATGTACGGCTCTGCATGGTCTTCCAT
CLCd-F	TACTCATCGGGATCGGAACTGGATTAG
CLCc-R	GACTCTGAGCACTGAACTCTCGCATTG
CLCe-F	AAGCAGGAGACTGATCAGGATGAGGTT
CLCe-R	GTACATCGAGATAATGCCAACGAGACC
CLCf-F	TCATACGACCTGAAGTCTGCTGCAGAG
CLCf-R	GTCGTTGATCGATCTCCTACGACTCAT
CLCg-F	AAGCAGGATTGGAGAGGTCGTAGTAAG
CLCg-R	CGTGTAGAACAGGAGCTGCAGCTAGTG
ACTIN-F	GGCCGATGGTGAGGATATTCAGCCACTTG
ACTIN-R	TCGATGGACCTGACTCATCGTACTCACTC



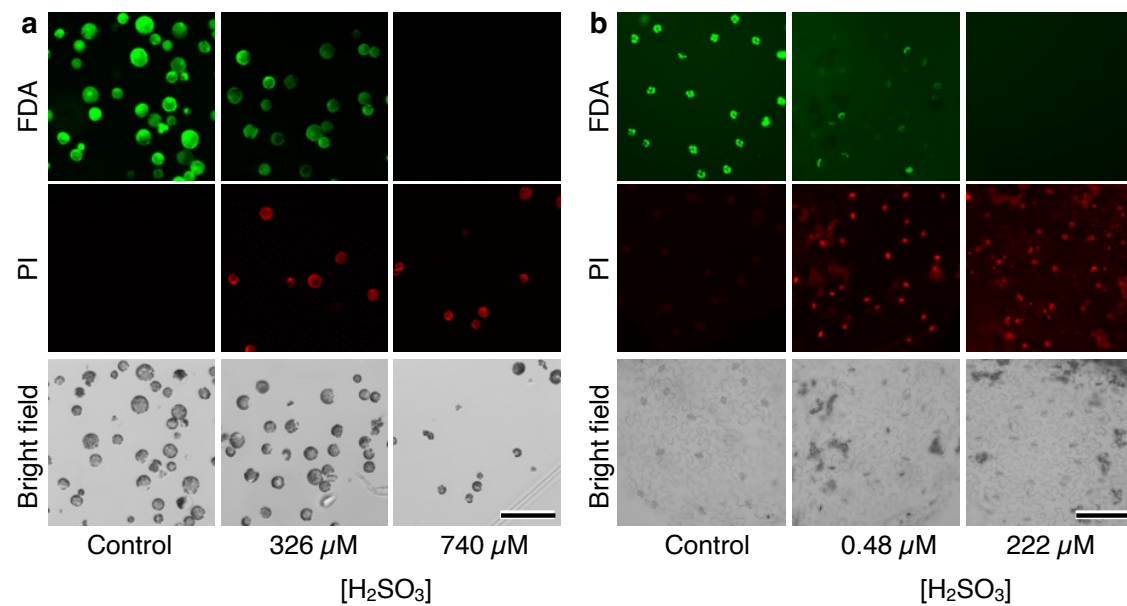
Supplementary Figure S1. Characterization of *clca3* mutant. Analysis of *CLCa* mRNA level, shoot fresh weight and nitrate content in shoot of plants grown for 6 weeks on Jiffy® peat pellet. The star indicates a significant difference (*t*-test followed by Bonferroni comparison post-test, $P < 0.05$)



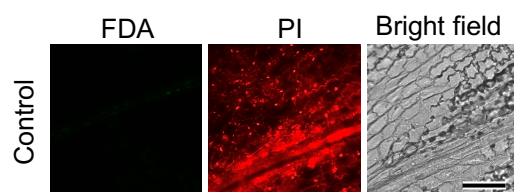
Supplementary Figure S2. Preparation of abaxial cell layer specimen of Arabidopsis petal by the tape-peel method.



Supplementary Figure S3. Staining of *Arabidopsis thaliana* petal tissue with FDA/PI. (a) Bright-field image of a cross section of a petal. (b-c) FDA/PI double-stained petal adaxial cell layer. (d-e) FDA/PI double-stained petal abaxial cell layer. (b and d) Control without boiling. (c and e) Boiled at 100 °C for 5 min. (b-e) Merged images of FDA fluorescence, PI fluorescence and bright field. (f) Typical fluorescence images of H₂SO₃-treated petal abaxial layer cells. Cell walls are stained by PI. PI staining of the control showed a few damaged cells. Scale bars = 100 μm.



Supplementary Figure S4. Typical fluorescence images of fluorescein diacetate (FDA)/propidium iodide (PI) staining of isolated mesophyll cell protoplasts and purified epidermis. (a) mesophyll cell protoplasts. Scale bar = 100 μm . (b) GC-enriched epidermal fragments. Scale bar = 50 μm .



Supplementary Figure S5. Adverse effect of *N*-acetylcysteine on petal abaxial layer cells. Petal abaxial layer was stained by FDA/PI double staining in the presence of 1 mM *N*-acetylcysteine. This treatment was done in the absence of H_2SO_3 (Control). Scale bar = 100 μm .