Calcium sensing receptor regulates stomatal closure through hydrogen peroxide and nitric oxide in response to extracellular calcium in Arabidopsis

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Supplementary Figures S1-S7

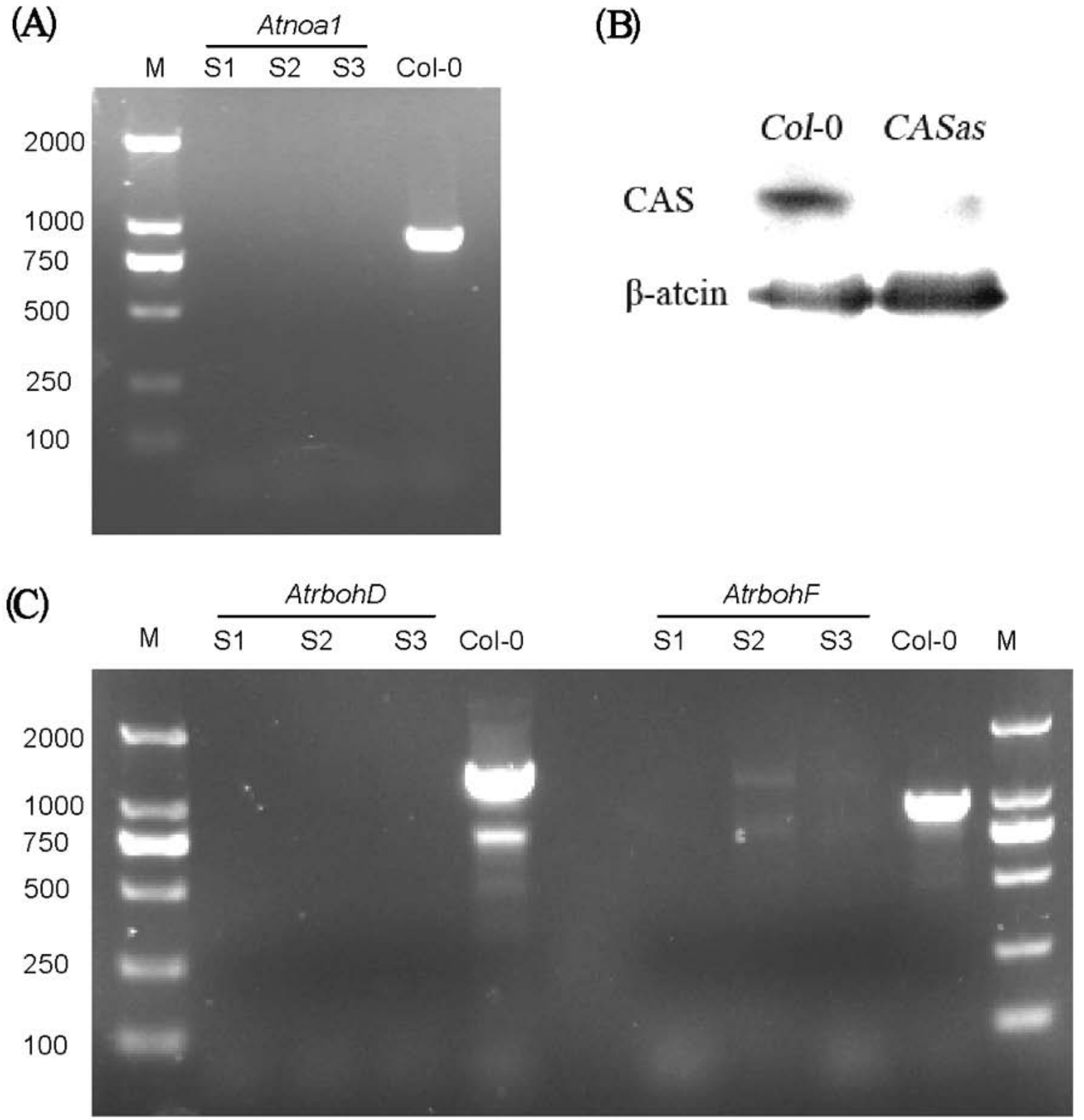

Fig. S1. Verifying the mutants using PCR and western blot. (A) Three independent samples of *Atnoa1* (S1 to S3) were confirmed by PCR analysis. (B) *CASas* was identified by western blot analysis using anti-CAS antibody. (C) Three independent samples of *AtrbohD/F* (S1 to S3) were confirmed by PCR analysis. The wild type (Col-0) was used as the control.

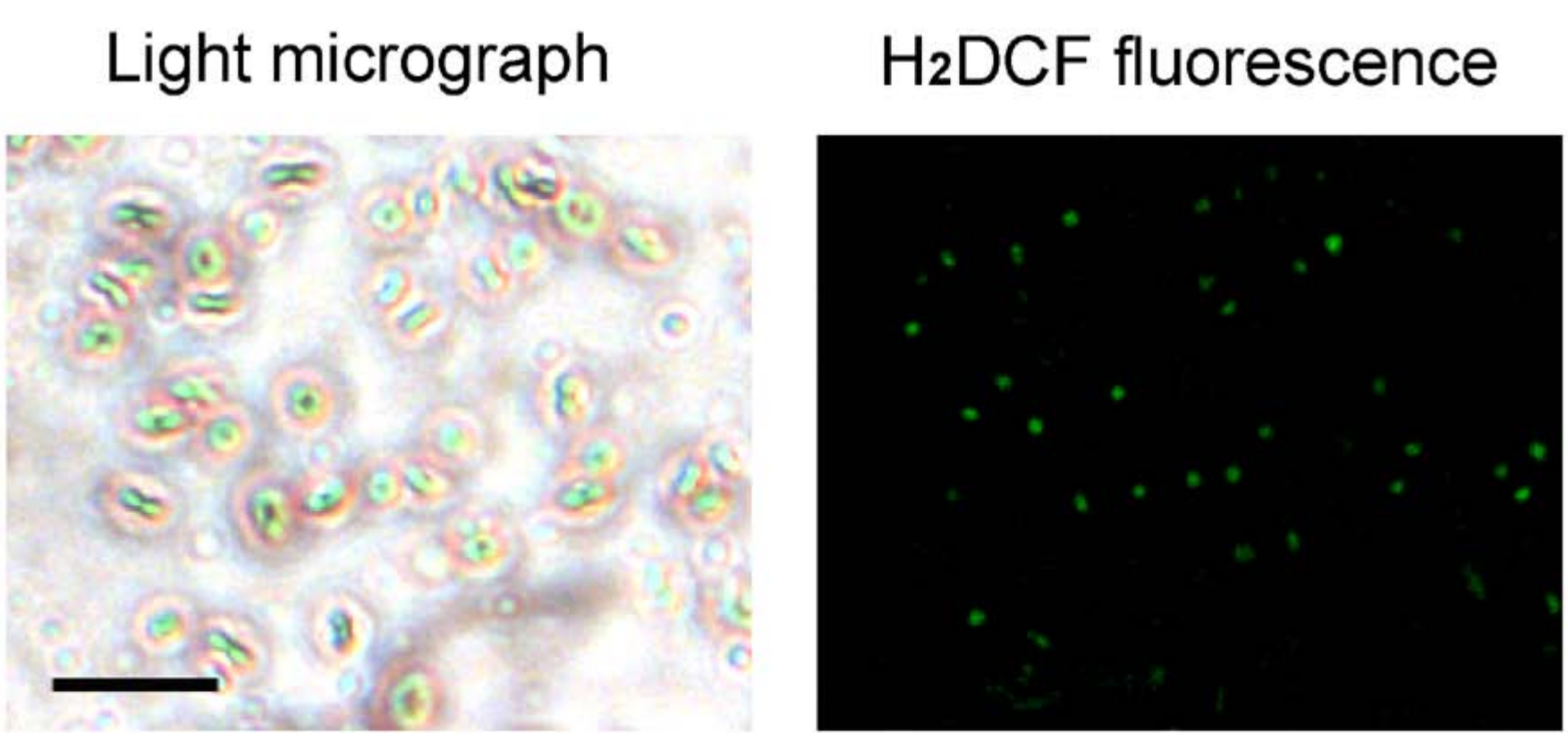

Fig. S2. Integrity of isolated chloroplasts was estimated by H_2DCF fluorescent dye. Isolated chloroplasts was incubated in HMS buffer containing 50 μM H_2DCFDA for 1 h at 4 °C in the dark, then rinsed twice by centrifugation at 2500 g for 4 min each in HMS buffer. Fluorescence from chloroplasts was observed by fluorescence microscope. Fluorescence dot was observed from over 85% of isolated chloroplasts. Bar=10 μm.

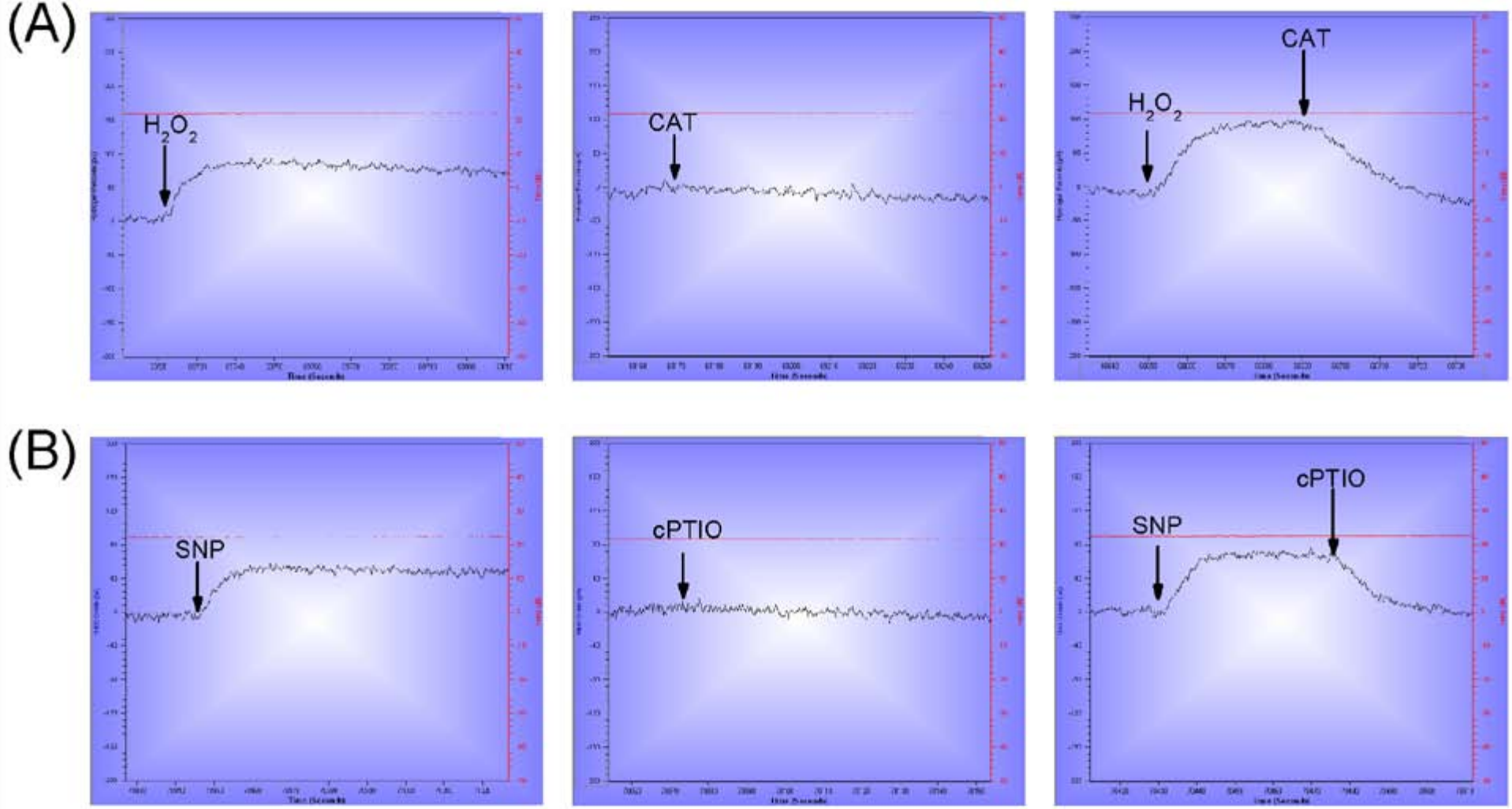

Fig. S3. The effects of CAT or cPTIO on H_2O_2 or NO current detected by H_2O_2 or NO selective electrochemical sensor. (A) Effects of CAT on H_2O_2 current detected by ISO-HPO sensor. 4 μM H_2O_2 or 100 units mL^{-1} CAT was added at the time point indicated by arrows. (B) Effects of cPTIO on NO current detected by ISO-NOP sensor. 1 μM SNP or 100 μM cPTIO was added at the time point indicated by arrows.

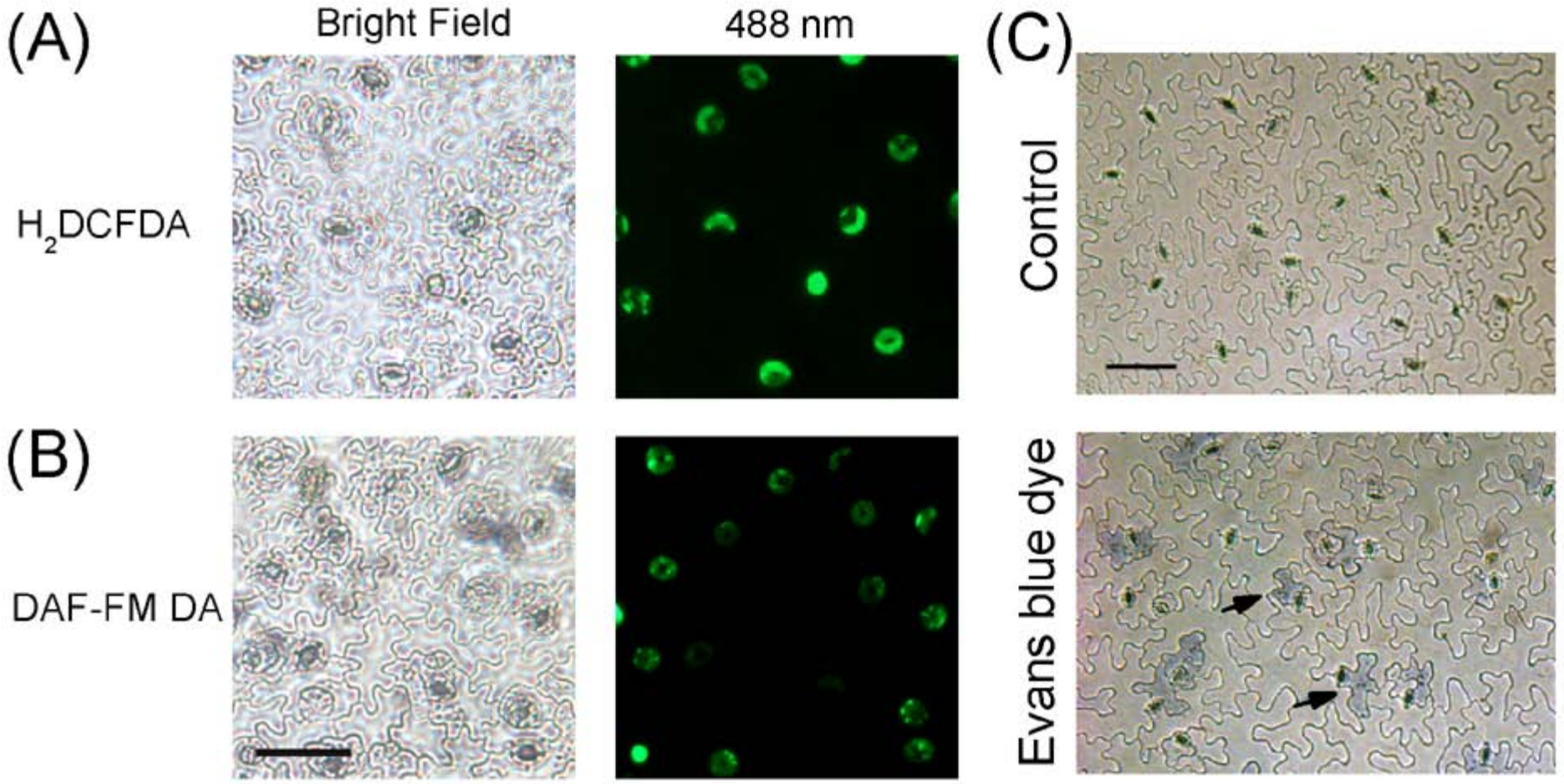

Fig. S4 Localization of H_2O_2 and NO on Arabidopsis epidermal peels in response to Ca^{2+}_o.

(A) H_2O_2-induced fuorescence was observed mainly in guard cells on epidermal peels exposed to 2 mM $CaCl_2$ in MES buffer for 20 min. (B) NO-induced fuorescence was observed mainly in guard cells on epidermal peels exposed to 2 mM $CaCl_2$ in MES buffer for 20 min. Bar = 100 μm. (C) Dead epidermal cells stained with Evans blue (shown by arrows) were indicated on the epidermal peels. Bar = 100 μm.

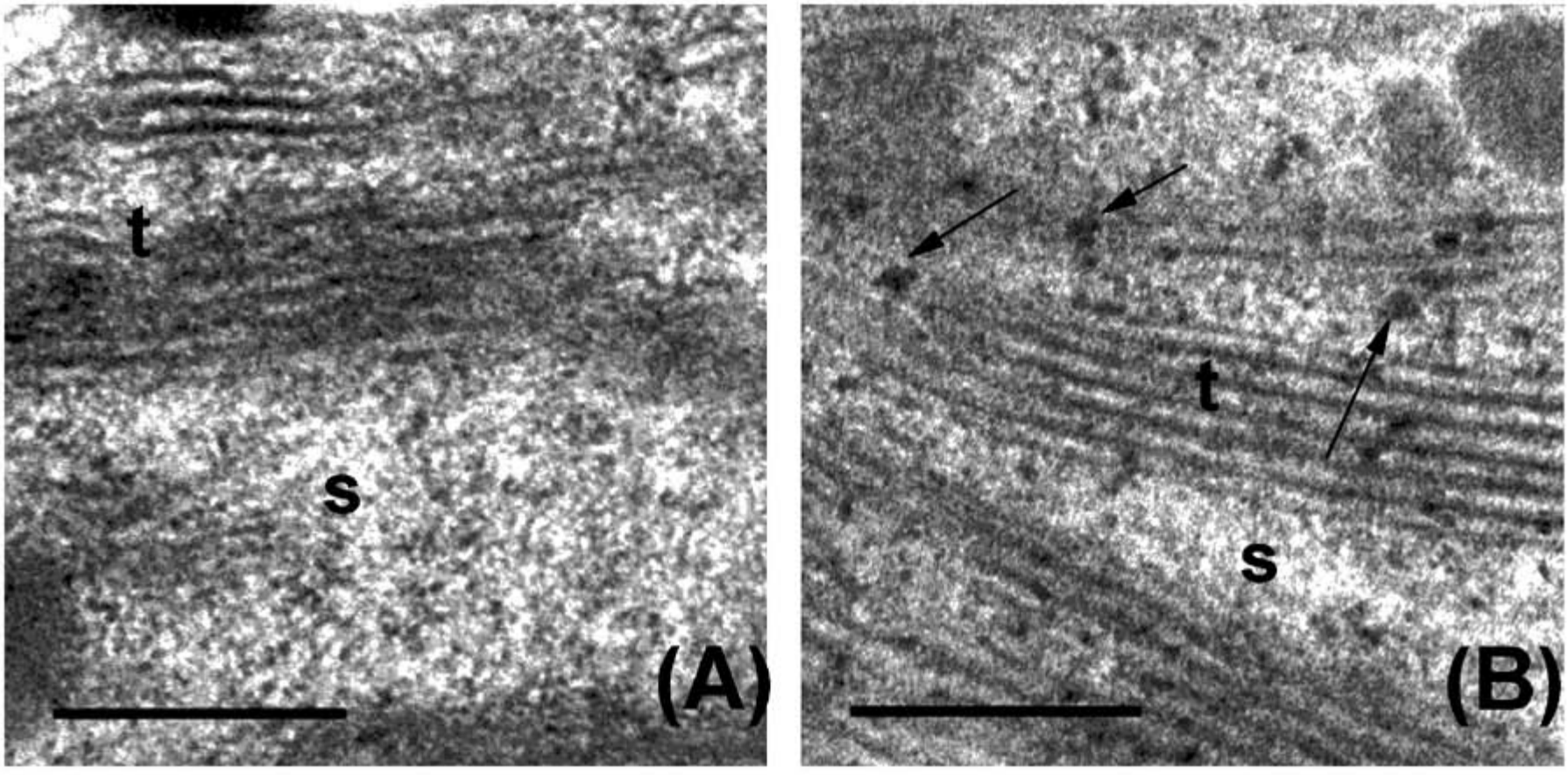

Fig. S5. ABA-induced H_2O_2 accumulation in Arabidopsis guard cell chloroplasts from the wild type with $CeCl_3$-staining by the TEM. (A) Guard cell from leaf section incubated in MES buffer (control) showed no H_2O_2 staining in chloroplast. (B) 10 μM ABA-induced H_2O_2 accumulation can be seen as black precipitate spots in chloroplast. The black spots in the transmission electron microscope images represent H_2O_2 forming electron dense cerium perhydroxide precipitates. Examples of individual precipitates are shown by arrows. s, stroma; t, thylakoids. Bar = 200 nm.

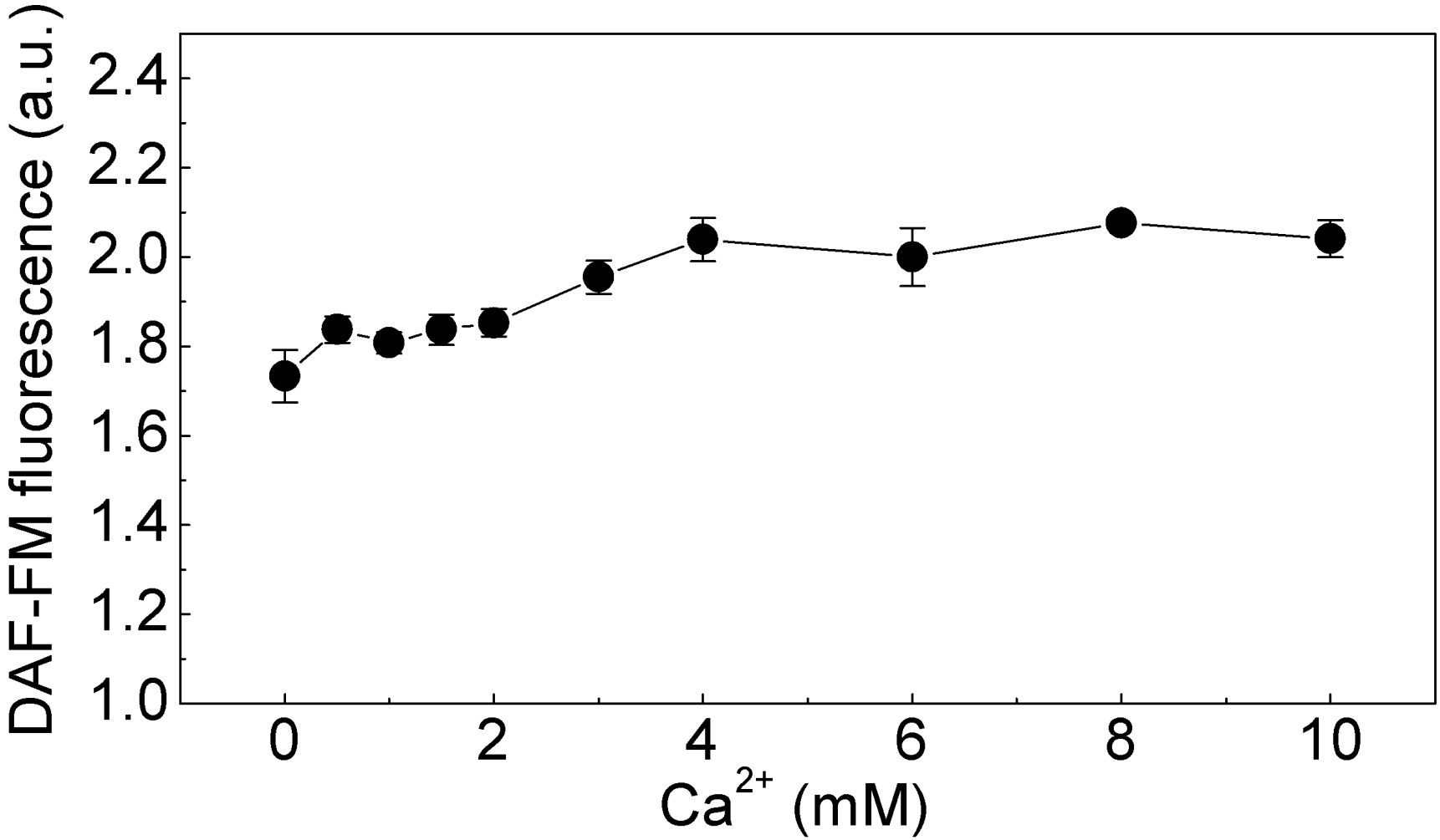

Fig. S6. Effect of Ca^{2+} on NO generation in isolated Arabidopsis chloroplasts. Averaged increases in relative DAF-FM fluorescence in chloroplasts plotted as a function of applied $CaCl_2$ for 10 min (n=8, ±SE).

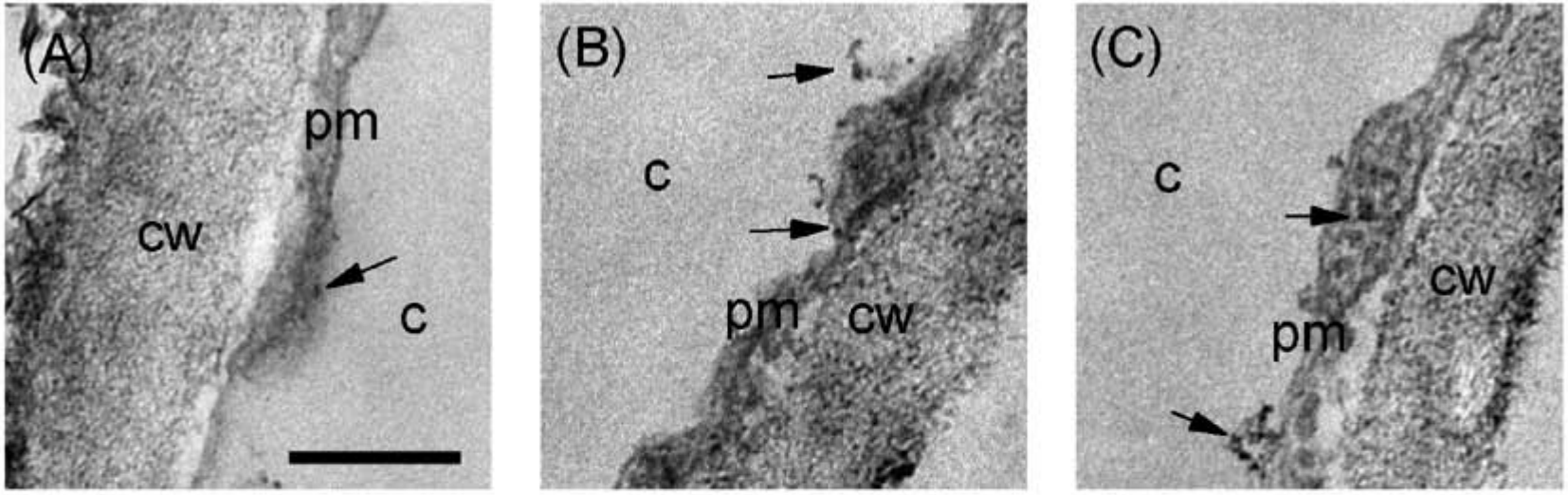

Fig. S7 TEM imgages of plasma membrane and cytoplasm in Arabidopsis guard cell with $CeCl_3$-staining in response to Ca^{2+}_o. (A) TEM images showing the plasma membrane and cytoplasm of the wild type guard cell with $CeCl_3$-staining without $CaCl_2$ treatment. (B, C) TEM images of plasma membrane and cytoplasm in guard cell with $CeCl_3$-staining from the wild type (B) or *CASas* (C) leaves incubated in 2 mM $CaCl_2$. The black spots in the TEM images represent H_2O_2 forming electron dense cerium perhydroxide precipitates. Examples of individual precipitates are shown by arrows. c, cytoplasm; cw, cell wall; pm plasma membrane. Bar = 200 nm.